COLOR STABILITY OF BLACK SORHGUM BRAN EXTRACTS UNDER DIFFERENT CONDITIONS

A Thesis

by

ANA PAOLA CARDENAS HINOJOSA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2008

Major Subject: Food Science and Technology

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Approved by:

Chair of Committee,	Lloyd W. Rooney
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ABSTRACT

Color Stability of Black Sorghum Bran Extracts Under Different Conditions.

(December 2008)

Ana Paola Cárdenas Hinojosa, B.S., Instituto Tecnologico y de Estudios Superiores de Monterrey

Chair of Advisory Committee: Dr. Lloyd W. Rooney

The color stability of 3-deoxyanthocyanins extracted from Tx430 Black and Black tannin sorghum brans were exposed to different pHs, temperatures, water activities, and concentrations for comparison with synthetic colorants Red No. 3, and Red No. 40 for 13 weeks. Dry extracts were solubilized using 0.5% citric acid in 70% aqueous ethanol. The color parameters of lightness, chroma, and hue of samples and synthetic standards were measured at pH ranging from 1 to 11; temperatures of -8, 4, 25, and 50°C; water activities of 1.00, 0.95, 0.85, and 0.20; and extract concentrations of 0.5, 1.0, 5.0, 10, 20 and 40 mg/mL for Tx430 Black and 0.1, 0.5, 1.0, 5.0, 10, and 20 mg/mL for Black tannin sorghums.

The absorbance values for thermal stability of the pigments from different pH levels of both black sorghum extracts were stable over time, especially at pH levels above 3. Lightness, chroma, and hue of Black PI Tall sorghum bran extracts at all pHs were more stable over time than those from Tx430 Black. The

Black PI Tall extracts were red, and closer in color to synthetic Red No. 40, while the orange colored extracts of Tx430 Black were more similar to those from synthetic Red No. 3. Color of both sorghum bran extracts and Red No. 40 were stable at all temperatures except 50℃; however, Red No.3 color was unstable at all temperatures over time. As water activity and extract concentrations of both sorghum brans increased, the color became more red. At low extract concentrations, the color of both black sorghum bran extracts was similar to synthetic Red No. 3, i.e. orange.

Sorghum bran extracts had better stability than natural fruit and vegetable anthocyanins. The condensed tannins present in Black tannin sorghum enhanced the stability of its extracts.

Overall, Black PI Tall and TX430 Black sorghum bran extracts have excellent potential for use in the food industry as natural colorants with significantly more stability than other natural pigments. The sorghums can be grown, stored and processed easily into bran concentrates which should reduce their cost and availability.

DEDICATION

I wish to dedicate this thesis to my parents Rafael and Bertha, my family, friends, and loved ones who gave me their support, their love, their humor, and most of all their patience as I tackled this project.

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There are numerous people I wish to thank for their help as I worked on this thesis. Included at the top of that list are my committee members. Dr. Lloyd Rooney was always cheerful while guiding this project. His academic and life support could never be fully repaid. I thank Dr. Joseph Awika for being there when I most needed him. Dr. Luis Cisneros-Zevallos, I thank you for your time, for your suggestions, for your corrections, and for your support. I thank Dr. Sergio Roman Othón Serna Saldivar who inspired me into the research and always encouraged me. I would like to express my sincere appreciation to Linda Dykes for her time, advise, knowledge and her willingness to share it. I thank Cassandra McDonough for her support, guidance, and understanding. Thanks to Dr. Ralph Waniska who was a great example of life.

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However, no one deserves more acknowledgment than my family, especially my mom and dad. Without their unwavering support and love, I would not be who I am today. They are the inspiration in my life.

NOMENCLATURE

min	minutes
g	grams
h	hours
mg	milligrams
μg	micrograms
mL	milliliters

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CHAPTER I

INTRODUCTION

Sorghum (*Sorghum bicolor*) is a major staple grain crop in Africa and Asia. Sorghum has great tolerance to dry, hot climates, which makes it a good crop that can be used as feed, food, and for fuel production. Sorghum is the fifth most important crop in the world after wheat, rice, corn, and barley. In 2007, the total production of sorghum in United State was 504,993 (1,000 Bushels) (United States Department of Agriculture-National Agricultural Statistics Service, 2008 data). More than 35% of sorghum is used for human consumption and the rest is used for animal feed and alcohol production (Awika & Rooney, 2004).

Until the middle of the nineteenth century, the colorants used in foods were of natural origin, from animals, plants, and minerals. In 1856, Sir William Henry Perkin discovered the first synthetic colorant and it drastically changed the food industry (Francis, 1999).

The use of synthetic colorant has been controversial because of safety issues and consumer perception. Alternatively, the use of natural colorants has been considered; however, this represents a challenge due to the low stability of natural colorants compared to the high stability of synthetic colorants (Cevallos-Casals et al., 2004).

This thesis follows the style and format of Cereal Chemistry.

Anthocyanins are natural pigments that provide color ranging from pink to violet. The pH, concentration, temperature, light, and, presence of co-pigments are some factors that affect the color stability.

Unlike the common anthocyanins found in fruits and vegetables, sorghum 3-deoxyanthocyanins do not have the hydroxyl group at position 3 of the C-ring making them more stable at high pH (Dykes et al., 2005; Awika, et al., 2004a). This property in addition to possible health benefits (i.e. antioxidant activity) makes sorghum 3-seoxyanthocyanins a potential source of a natural colorant in food systems.

Little work has been done on the stability of 3-deoxyanthocyanins so there is a need to study their stability and to compare them with the stability of synthetic colorants.

Objectives

The aim of this study was to investigate the color stability of 3-deoxyanthocyanins from black sorghum brans relative to synthetic colorants when they are exposed to different conditions.

The specific objectives were:

1. To evaluate the effect of pH, temperature, pigment concentration, and water activity on the color stability of 3-deoxyanthocyanins from black sorghum bran.
2. To compare the stability of the 3-deoxyanthocyanins from sorghum with FD&C Red No. 3 and FD&C Red No. 40.

CHAPTER II

LITERATURE REVIEW

Colorants

Color is one of the most important quality attributes of foods (Fennema, 1996). This attribute along with appearance are the first to be evaluated by the consumer when they select foods. The consumer relates specific colors of foods to quality. Natural and processed foods have a characteristic color that consumers identified and any change can modify the acceptance of the food (Badui, 1999). Color also influences flavor perception. Consumers relate colors with some flavors, i.e. they expect red color in a candy or drink to be strawberry or cherry flavored (Francis, 1999).

By the time processed food gets to the consumer, the color could change, becoming unappealing. Manufactures in the food industry enhance the color of foods with synthetic colorants or natural pigment extracts (Bridle & Timberlake, 1996; Guisti & Wrolstad, 2003).

A colorant is any chemical, either natural or synthetic, that imparts color. Foods have color because of their ability to reflect or emit different quantities of energy at wavelengths able to stimulate the retina in the eye. The eye can perceive the color when the energy is in a wavelength range between 380 and 770 nm (Hendry & Houghton, 1992). However, not all people perceive color the same way.

Identification of colorants is based on their properties to absorb certain wavelength in the visible region. Besides absorbance property, there are other methods that help to measure color, like colorimetric systems, based on uniform color spaces (CIELUV and CIELAB) and non-uniform color spaces (CIEXYZ) and other methods that compare the color with standard colors (Montes et al., 2005).

Human eye senses six hues: red light around 700nm, orange at 625 nm, yellow at 600 nm, green at 525 nm, blue at 450 nm, and violet at and below 400 nm. Pigments absorb light at various wavelengths; the remaining colors are perceived by the human eye as a distinct color (Hendry and Houghton, 1992). Table I shows the absorption of some colors at a specific wavelength that could be perceived as a different color by the human eye.

Table I Perception of color according to the wavelength of light absorbed

Color absorbed	Red	Orange	Yellow	Yellow -green	Green	Green -blue	Blue	Violet
Absorption wavelength max (nm)	675	600	585	570	540 525	490	460	410
Color perceived	Blue-green	Blue	Violet	Mauve Red	Orange	Yellow	Yellow -green	

Source: Data from Hendry and Houghton. (1992)

Synthetic colorants

The use of colorants in the United State is controlled by the 1960 Color Additive Amendment to the U.S. Food, Drug and Cosmetic Act of 1938. Certified colorants are synthetic dyes that are not found in nature and meet specific government quality standards. Colorants exempt from certification are natural pigments or specific synthetic dyes that are identical to natural colorants (FDA, 2007).

The type and amount of colorants permitted in food varies among countries. A list of the nine certified colors approved for use in the United States is presented in table II.

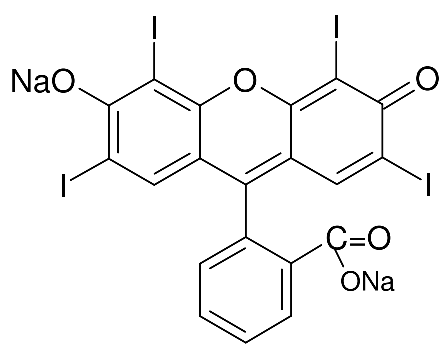
Table II Color additives approved for use in human food in U.S.

Federal Name	Year approved	Hue
FD&C Blue No. 1	1969	Greenish blue
FD&C Blue No. 2	1987	Deep blue
FD&C Green No. 3	1982	Bluish green
Orange B	1966	
Citrus Red No. 2	1963	
FD&C Red No. 3	1969	Bluish Pink
FD&C Red No. 40	1971	Yellowish red
FD&C Yellow No. 5	1969	Lemon yellow
FD&C Yellow No. 6	1986	Redish yellow

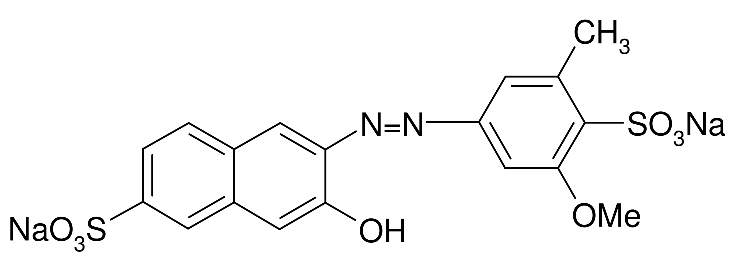
Source: Data from Francis. (1999)

Colorants behave differently when incorporated in food systems. One of the most popular colors in foods is FD&C Red No. 40. The daily intake of this synthetic color is 100 mg/day (Giusti & Wrolstad, 1996; Torres Ochoa, 2002).

The structure of the synthetic colorant FD&C Red No. 3 and FD&C Red No. 40 are present in Fig. 1. The Synthetic colorant Red No. 3 is also known as Erythrosine and is a cherry-pink coal-base dye. Synthetic Red No. 40 is an azo dye also knows as Allura Red. The presence of water-solubilizing substituents like SO_3 groups gives the advantage to be more stable over a wide pH range (Zollinger, 2003).



FD&C Red No. 3



FD&C Red No. 40

Fig. 1. Structure of synthetic colorants FD&C Red No. 3 and FD&C Red No. 40.

The chemical structure has an effect on the stability of the colorants. Table III shows the stability of FD&C Red No.3 and FD&C Red No. 40 under different conditions.

Table III Stability of FD&C Red No. 3 and FD&C Red No. 40 under various conditions

Colorant Name	Conditions				
	Light[a]	**Acids[a]**	**Alkalies[a]**	**Heat[a]**	**10% citric acid[b]**
FD&C Red No.3	2	2	5	5	ins
FD&C Red No. 40	5	5	4	3	naf

Source: Data from Francis. (1999)
[a] Stability: 1 = very poor, 5 = good
[b] ins = insoluble, naf = no appreciable fading

Natural pigments

Consumers are concerned about the safety of synthetic colorants in foods and often prefer natural pigments. Humans have used natural colorants since prehistoric times. They dyed furs, and other objects using natural pigments from vegetables and animals. Natural pigments are either organic or inorganic. Organic pigments have higher tinctorial strength and brightness than inorganic. However, their thermo- and photo-stabilities are lower. Inorganic are opaque while organic are more transparent (Zollinger, 2003).

It has been difficult to find a natural red colorant that replaces FD&C Red No. 40. Some natural colorants have been evaluated in different food systems; but they do not achieve the desirable hue (Giusti & Wrolstad, 1996). The cost of extraction is high, they may impart some odors, and have relatively low stability to processing, formulation and storage conditions (Giusti and Wrolstad, 2003; Reyes & Cisneros-Zevallos, 2007). Synthetic colorants are highly stable to light, oxygen, temperature, pH, and other factors, making their replacement with natural pigments complicated.

Measurement of Color

Some visual comparisons of color like the Munsell system have been developed. These are useful particularly for grade standards, but they are available in limited number of colors, and they may change color when exposed to light. Thus, instrumental methods of color measurement have been developed (Francis, 1999).

The development of instrumental methods of color measurement was based on reflection or transmission. There are two basic instruments, spectrophotometer and colorimeters. Spectrophotometer is the most fundamental instrument for color measurement. It measures the light reflected or transmitted from the materials as a function of wavelength (Berns, 2000). Colorimeters are designed to give the tristimulus values of materials directly and they duplicate the response of the human eye. They use a light source, three glass filters with transmittance spectra that duplicate the X, Y, and Z curves, and a photocell to read the color of the sample (Christie et al., 2000).

The XYZ system, Judd-Hunter La*b* system, and the CIELCH system have been used in colorimeter measurements. The XYZ system is referred to as the 1931 standard observer or the 2° observed and is assumed to represent the color matching results of the average of the human population having normal color vision (Berns, 2000). This system was used by early researchers to describe color in terms of (X) red, (Y) green, and (Z) blue. The problem of the system is the location of black that is not well defined. The Judd-Hunter La*b* system is usually called CIELAB (CIE = Commission International de l'Eclairage). This system measures L which is a measure of lightness (100) or darkness (0) and two coordinates a* and b*. Positive and negative a* values indicate redness and greenness, respectively. Positive and negative b* values indicate yellowness and blueness, respectively. The CIELAB system can be used for color difference changes during processing and storage (Vatai et al., 2008). L, a*, and b* coordinates are not independent of each other; a* and b* are related to each other and both are dependent on L.

For better description of color changes, La*b* are sometimes converted to the LCH system. The CIELCH system is easier to correlate with the visual appearance of a color and was developed as a description of color tolerance (Ohta & Robertson, 2005). This system has the advantage that color differences can be determined with the aid of relatively simple computer programs and expressed in terms of lightness-difference (ΔL), chroma-difference (ΔC), and hue-difference (ΔH) (Zollinger, 2003). L is absolute white at 100 and absolute black at 0; chroma (C) is a measure of the intensity or saturation and is calculated as $(a^* + b^*)^{½}$. Hue (h) is the angle derived from the two coordinates a* and b* and is calculated as arc tan (b*/a*). Hue angles are expressed in degrees from 0° to 360°, where 0° is on the +a* axis, rotating anti-clockwise to 90° for the +b* axis, 180° for –a* axis, and 270° for –b* axis (Duangmail, et al., 2007; Vatai, et al., 2008).

The light source is critically important for the correct description of color. There are two illuminant conditions that can be used in the colorimeter for the calculations of color coordinates: D_{65} illuminant is the typical diffuse daylight (predominance of short wavelengths) that represent color temperature of about 65000 K and CIE illuminant A represents an incandescent lamp (predominance of long wavelengths) (Gonnet, 1998).

The most common way to indicate anthocyanin color is based on visible λ_{max} values from UV/Vis absorption spectra. Anthocyanins in acid solutions occur in flavylium forms so the spectrophotometer can measure the color. However, when the pH increases, anthocyanins occur as a mixture of different equilibrium forms. Thus, the spectrophotometer does not give accurate color measurements at high pH. In those cases CIELAB system which measure the absorption/transmission at all wavelengths, is preferred (Torskangerpoll & Andersen, 2005).

Anthocyanins

Flavonoids are naturally occurring substances characterized by a C_6-C_3-C_6 carbon skeleton. They are present without sugar as agylcones or with sugar as glycosides (Peterson & Dwyer, 1998). Flavonoids include flavanols, flavanones, flavones, flavonols, and anthocyanins (Fig. 2). Unlike other flavonoids, anthocyanins strongly absorb visible light and they generate an infinite variety of colors depending on the media in which they occur.

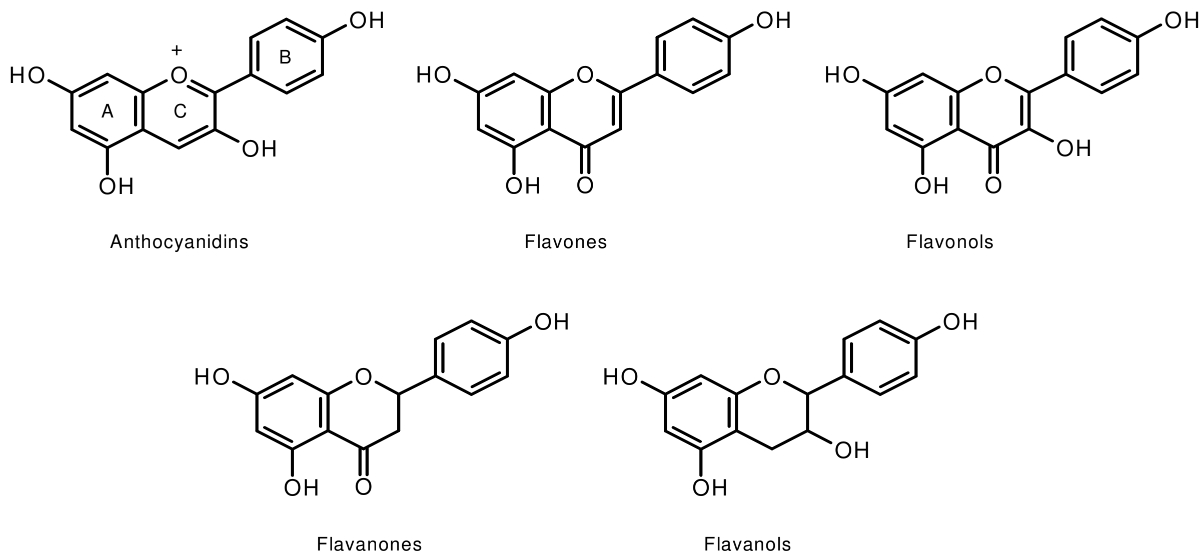


Fig. 2. Structure of flavonoids.

The most important water-soluble pigments are the anthocyanins. They impart the red-purple to blue colors in fruits, vegetables, and cereal grains (Clifford, 2000; Wrolstad, 2004). Anthocyanins are glycosylated polyhydroxy and polymethoxy flavylium salts. The main part of an anthocyanin is its aglycone, the flavylium cation (Prodanov, et al., 2005; Rein, 2005).

Anthocyanins differ in the number of hydroxyl and/or methoxyl groups present; the types, numbers, and sites of sugars attached to the molecule; and the types and numbers of aliphatic or aromatic acids that are attached to the sugars on the molecule (Mazza & Miniati, 1993; Fennema, 1999; Clifford, 2000).

Anthocyanins as colorants

Anthocyanins contribute color and hence the acceptability of food products. They are water-soluble and have been consumed without negative effects (Giusti & Wrolstad, 2003; Yawadio & Morita, 2007). Anthocyanins range from purplish red to a red nearly equivalent to FD&C Red No.40. The daily anthocyanin intake was estimated at 215 mg/day during the summer (Bridle & Timberlake, 1996; Clifford, 2000). In the United States, anthocyanins are permitted as food colorants under the category of fruit or vegetable juice color.

A free hydroxyl at the 5, 7 or 4’ positions is essential for generating the *in situ* colors responsible for plant pigmentation. In aqueous media, most of the anthocyanins behave like pH indicators; at low pH they are red, colorless at intermediate pH, and blue at high pH (Mazza & Miniati, 1993).

The anthocyanin color is a result of the excitation of the flavylium cation by visible light. The flavylium cation contains conjugated double bonds, which are excited very easily; their presence is essential for color. Increasing substitution on the flavylium cation gives a deeper hue which depends on the bathochromic change. This means that the light absorption band in the visible spectrum shifts from violet to red to blue. Hyperchromic change is a shift from blue to red to violet. Auxochromes are electron-donating groups that, by themselves, have no chromophobic properties. When they are attached to the flavylium molecule, they have a deeper hue. In anthocyanins auxochromes are hydroxyl and methoxyl groups (Fennema, 1996; Rein, 2005). However, other factors like change in pH, copigmentation, and metal complex formation affect hue.

Besides the color attributes, anthocyanins are non-toxic and non-mutagenic with positive health benefits. Some benefits include antioxidant capacity, treatment of various blood circulation disorders, anti-inflammatory properties, and risk reduction of coronary heart diseases and cancer (Hollman et al., 1996; Clifford, 2000; Giusti & Wrolstad, 2003).

Color stability of anthocyanins

The use of anthocyanins as a colorant in food products is preferred over synthetic colorants, but they are highly unstable. Degradation occurs during extraction, purification, processing, and storage. Color stability of anthocyanin pigments depend on structure and concentration of anthocyanins, pH, temperature, light, oxygen, solvents, enzymes, ascorbic acid, sugar, and other substances (Cevallos-Casals & Cisneros-Zevallos, 2004; Sadilova et al., 2006).

Structure of anthocyanins

Some anthocyanins are more resistant to degradation because they contain hydroxyl, methoxyl groups, sugars, and acylated sugars (Mazza & Miniati, 1993; Rein, 2005).

Anthocyanins change color from orange to blue when the number of hydroxyl groups increases. The visible absorption changes to longer wavelengths. Methoxyl groups reverse the change. Presence of a hydroxyl group in the C3 position changes the color from yellow-orange to red. This explains the difference between the anthocyanins that are red and the 3-deoxyanthocyanins that have a yellow color (Mazza & Miniati, 1993). The absence of the hydroxyl group makes the 3-deoxyanthocyanins more stable.

pH

pH affects the color and stability of anthocyanins. Anthocyanins change color from red to blue-red, purple, and blue and sometimes from green to yellow when the pH is increased. Color intensity is also pH-dependent, being more intense at pH 1.0 and decreasing as pH increases. Anthocyanins are more stable in acidic pH than in neutral or alkaline pH (Markakis, 1982).

Temperature

Increasing the temperature during processing and storage increases the degradation rate of anthocyanins and reduce the color intensity.

Anthocyanin structure, pH, the presence of oxygen, and the interaction with other components affect thermal degradation. Anthocyanin structures with increased pH stability also have increased thermal stability (Hendry & Houghton, 1992).

Markakis (1982) suggests that the first step of degradation is the formation of a chalcone glycoside; further degradation forms brown products, especially if oxygen is present.

Light

Anthocyanins are unstable when exposed to UV, visible light or other sources of ionizing radiation. The end-products of light-induced degradation of anthocyanins are the same as in thermal degradation with differences in the kinetic pathway (Rein, 2005).

Oxygen

Oxidation together with temperature, pH, light, and/or ascorbic acid accelerates anthocyanin degradation. The two factors that provoke the most destruction of anthocyanins are oxygen and temperature. Degradation can be by a direct oxidation mechanism and/or by indirect oxidation where oxidized compounds react with anthocyanins, giving colorless or brown products. Precipitation and haze may result from direct oxidation (Hendry & Houghton, 1992).

When anthocyanins react with oxygen radicals, anthocyanins act as antioxidants. These reactions explain the health benefits of anthocyanin pigments against cardiovascular diseases (Rein, 2005).

Enzymes

Anthocyanases including glycosidases and polyphenol oxidases (PPO) cause discoloration of anthocyanins. Glycosidases hydrolyze glycosidic bonds yielding free sugars and aglycones. The decreased solubility of anthocyanins causes significant loss of color intensity. PPO oxidizes anthocyanins in the presence of oxygen. PPO catalyzes the oxidative transformation of catechol to quinones. Later, quinones react with each other, with amino acids or proteins to yield brown-colored polymers (Hendry & Houghton, 1992). Enzyme activity has to be inactivated if maximum pigment retention is desired. Anthocyanases are inactivated by heat (Markakis, 1982; Fennema 1996; Hendry & Houghton, 1992).

Ascorbic acid

Ascorbic acid is a natural antioxidant that affects color stability of anthocyanins. Crude 3-deoxyanthocyanin extract showed an unusual stability in the presence of 500 mg L^{-1} at low pH (Ojwang, L and Awika, J., 2008). However, in extreme conditions, such as radiation and high temperatures, the antioxidant properties disappear (Torres Ochoa, 2002). Ascorbic acid accelerates anthocyanin degradation which results indirectly from hydrogen peroxide that forms during oxidation of ascorbic acid. Other mechanisms are direct condensation between anthocyanins and ascorbic acid (Markakis, 1982).

Sugars

Sugars at high concentrations have a protective effect on anthocyanins by lowering water activity. However, sugars present at low concentrations have little effect on water activity and accelerate anthocyanin degradation. Glucose, sucrose, and maltose have less degradative effects on anthocyanins than fructose, arabinose, and lactose (Markakis, 1982; Fennema, 1992).

Copigmentation

Copigmentation plays a major role in the color expression of anthocyanin pigments in aqueous media (Gonnet, 1999). Copigmentation is defined as the phenomenon which makes the color of anthocyanins bluer, brighter, and more stable due to interaction between organic substances and anthocyanins (Osawa, 1982).

Co-pigmented structures cause a “bluing effect” because anthocyanins change from red to blue, with a strong increase in color intensity (hyperchormic effect) (Gonnet, 1998; Bąkowska et al., 2003).

Copigmentation improves the color and stability of anthocyanin-rich food products; the color can be stabilized and enhanced by the addition of different plant extracts rich in copigments.

Copigments are colorless or very slightly yellowish colored molecules occurring naturally alongside anthocyanins. The most common copigments are flavonoids, and other polyphenols, alkaloids, amino acids, and organic acids (Gradinaru et al., 2003). The addition of polyphenols to anthocyanin solutions as copigments increases anthocyanin stability during thermal processing and storage of model fruit juice systems. Thus, they could be used commercially to prevent color loss and phytochemical degradation of anthocyanin- and ascorbic acid- containing juices (Del Pozo-Insfran et al., 2007).

Copigmentation results from intermolecular and intramolecular complex formation, as well as self-association and metal complexing.

Intermolecular copigmentation of anthocyanins with other flavonoids produce an increase in color intensity (hyperchromic effect) and a shift in the wavelength of maximum absorbance toward higher wavelengths (bathocromic shift), giving purple to blue colors. The intensity of the copigmentation is dependent on several factors including type and concentration of anthocyanin, type and concentration of copigment, pH, and temperature (Mazza & Miniati, 1993; Mollov et al., 2007).

Intramolecular copigmentation is responsible for the color stability of anthocyanins containing two or more aromatic acyl groups (Mazza & Miniati, 1993). The covalent acylation of the anthocyanin molecule stabilizes the pigment. The factors that affect intramolecular copigmentation are the number of acyl groups, their structure, the position of the glycosyl residues, and the structure and number of saccharides (Rein, 2005). Due to the strengths of the bonds, intramolecular copigmentation is thought to be more effective in stabilizing anthocyanin colors than intermolecular copigmentation.

Self-association is when the color intensity of the anthocyanin increases more than linearly with an increase in pigment concentration. This phenomena also occurs in concentrated neutral solutions and is affected by the type and concentration of anthocyanin. But is still not clear whether self-association causes color stability, because at neutral solutions only a small amount of the pigment remains in the colored form (Mazza and Miniati, 1993).

Metal complexing is the formation of a complex between anthocyanin and a metal such a tin, copper, magnesium, and potassium, The flavylium is stabilized and do not form colorless pseudobase by hydration when it is dissolved in concentrated aqueous solution of neutral salts (Mazza and Miniati,1993).

Sorghum

Sorghum is the fifth most important cereal crop in the world after wheat, rice, corn, and barley (Perez, 2005).

The color of the pericarp is controlled by *R* and *Y* genes. These genes produce the white, yellow and red color of sorghum. When *Y* is recessive the pericarp is white. Recessive *R* and dominant *Y* genes produce yellow pericarp. Red pericarp is produced when both *R* and *Y* genes are dominant. The thickness of the pericarp is controlled by the *Z* gene. When the gene is recessive the pericarp is thick. On the other hand, when the gene is dominant the pericarp is thin (Rooney & Serna, 2000; Dykes & Rooney, 2006).

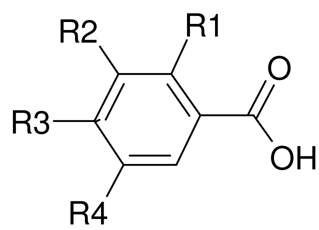
Sorghums that have tannins have a pigmented testa. The presence of pigmented testa is controlled by the B_1 and B_2 genes. These genes are dominant in sorghums with pigmented testa.

Based on the genetics and extractable tannin content, sorghums are classified into three types. Type I sorghums contain low levels of phenols and have no pigmented testa. Type II sorghums have a pigmented testa and tannins are extracted with acidified methanol because they are located in the vesicles within the testa layer and need a strong acid that can disrupt the structure of vesicles. Type III sorghums have pigmented testa and tannins are extracted in both methanol and acidified methanol. Tannins in type III are located in the cell walls of the testa and some in the pericarp (Awika & Rooney, 2004).

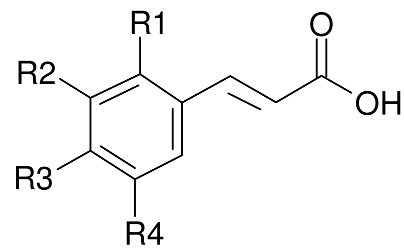
Phenolic compounds in sorghum

Sorghums contain phenolic compounds, which affect the color, appearance, and nutritional quality of the grain. The type and level depends on genetics and environmental conditions. There are three types of phenolic compounds in sorghum: phenolic acids, flavonoids and, condensed tannins. Phenolic acids are present in all sorghums. They are located in the pericarp, testa, aleurone layer, and endosperm. Sorghums also contain flavonoids and the ones with a pigmented testa contain condensed tannins (Dykes & Rooney, 2006).

There are two classes of phenolic acids (Fig. 3): benzoic acid derivatives, (e.g. gallic, syringic, p-hydroxybenzoic, vanillic, and protocatechuic acids) and cinnamic acid derivatives (e.g. coumaric, caffeic, ferulic, and sinapic acids). They are concentrated in the outer layers of the kernel and in the cell walls throughout the kernel. Ferulic acid is the most abundant phenolic acid and is present in bound form (Gous, 1989).



Benzoic acids



Cinnamic acids

Fig. 3. Structures of benzoic acids and cinnamic acids.

Phenolic acids have good antioxidant activity and may contribute to the human health benefits associated with whole grain consumption (Awika & Rooney, 2004).

There are two classes of tannins: hydrolysable tannins, which are esters of gallic or ellagic acid and glucose, and condensed tannins. Type II and Type III sorghums contain only condensed tannins also called proanthocyanidins or procyanidins (Fig. 4). These high molecular weight polyphenols are mainly polymerized favan-3-ols and/or flavan-3,4-diols, linked between flavonol subunits by carbon-carbon bonds with (-)-epicatechin chain extension unit and (+)-catechin as chain termination units. Tannins protect the sorghum kernel against attack of insect, birds, and microorganisms (Awika & Rooney, 2004).

The antioxidant activities of sorghums with a pigmented testa are higher than non-tannin sorghums and the bran provides higher values of antioxidant activity than some fruits. However, tannins bioavailability is still unknown; recent data suggest that the tannins are more bioavailable than previously thought (Awika & Rooney, 2004).

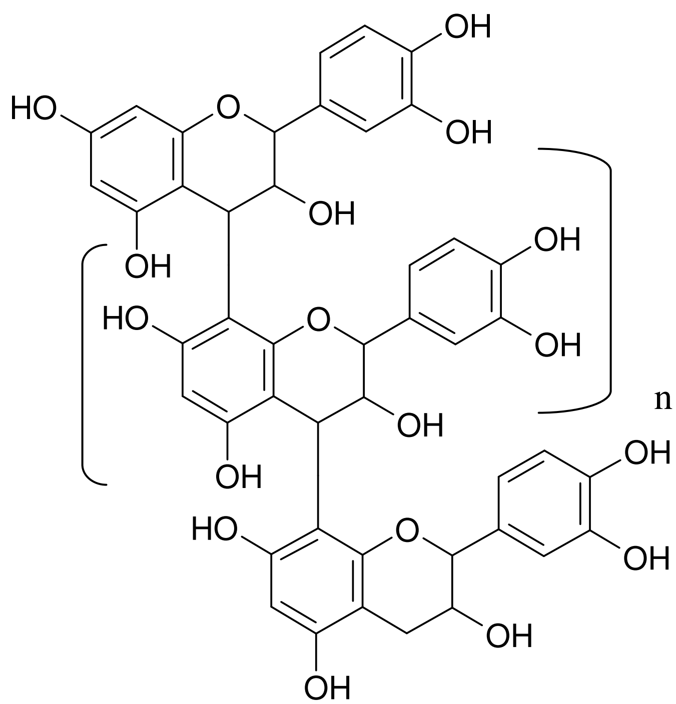


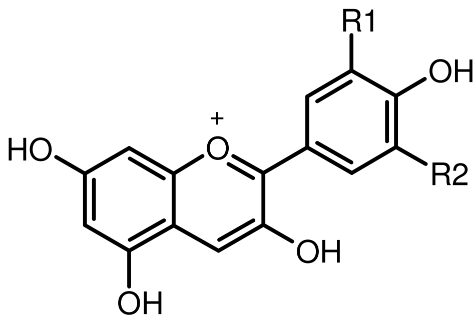
Fig. 4. Proanthocyanidin polymer.

Sorghum flavonoids

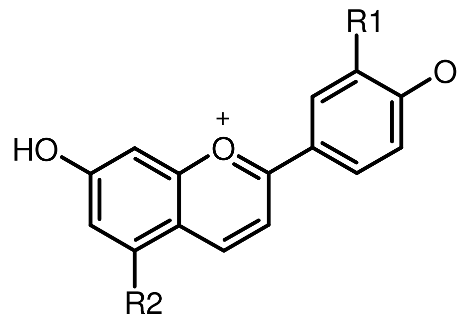
Flavonoids are naturally occurring substances in plants that are thought to have positive health benefits on humans. These benefits include antioxidant capacity, anti-inflammatory properties, and control of diabetes (Awika & Rooney, 2004).

The major class of flavonoids studied in sorghum is the anthocyanins. Anthocyanins in sorghum are unique. They are called 3-deoxyanthocyanins because of the lack of the hydroxyl group in the 3-position of the C-ring (Fig. 5). Sorghum is considered to be the only significant dietary plant source of this uncommon anthocyanin (Awika et al. 2004b; Awika & Rooney, 2004; Dykes & Rooney, 2006).

3-Deoxyanthocyanins are distinguished from the common anthocyanidins by: their distinctive color, chromatographic and spectral properties; their greater stability to oxidation; and, the failure of the aglycone to fade on chromatograms developed in solvents lacking mineral acid (Harborne, 1996).



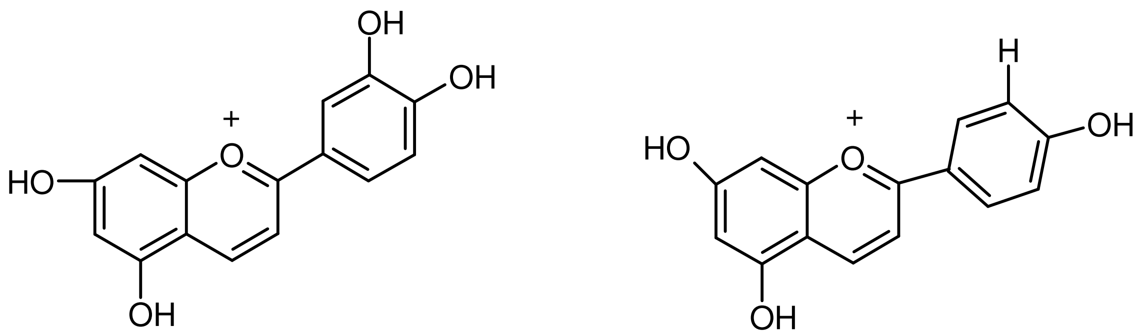
Anthocyanidins



3-Deoxyanthocyanidins

Fig. 5. Structure of anthocyanins and 3-deoxyanthocyanins.

The two common sorghum 3-deoxyanthocyanidins are: apigeninidin and luteolinidin (Fig. 6). The 3-deoxyanthocyanidins produce yellow (apigeninidin) and orange (luteolinidin) color in acidic solvents, making them different from the anthocyanins and their aglycones, which have a red to purple color at low pH. Other 3-deoxyanthocyanins include 5-methoxyluteolinidin, 7-methoxyapigeninidin, apigeninidin 5-glucoside, luteolinidin 5-glucoside, 5-methoxyluteolinidin 7-glucoside, 7-methoxylapigeninidin 5-glucoside, 5-methoxyapigeninidin, and 7-methoxyluteolinidin (Wu and Prior, 2005).



Luteolinidin **Apigeninidin**

Fig. 6. Structure of luteolinidin and apigeninidin.

Black sorghum

Black sorghums are genetically red, but during maturation exposure to sunlight turns the pericarp color from red to black (Dykes et al., 2005).

Black sorghums have the highest levels of 3-deoxyanthocyanins compared to brown and red sorghum. These compounds are concentrated in the bran; the levels are 3 to 4 times higher than the anthocyanins in grains. (Awika et al., 2004a)

Apigeninidin and luteolinidin are the main 3-deoxyanthocyanins present in black sorghums. They represent around 36-50% of the total anthocyanins identified by HPLC (Gous 1989; Awika et al., 2004b).

Gous studied the stability of Shawaya sorghum, which is an intensely black pigmented sorghum, finding that the 3-deoxyanthocyanins are more stable at pH values near neutrality than the common anthocyanins. At elevated temperatures, the crude pigment extract was less stable than the individual anthocyanidins luteolinidin and apigeninidin (Gous, 1989). But more research is needed to see the stability over time.

CHAPTER III

MATERIALS AND METHODS

Raw materials

Black sorghum brans

Two black sorghum brans were obtained: Tx430 Black (College Station, 2001) and Black PI Tall (College Station, 2006). Sorghums were decorticated using a PRL dehuller (Nutana Machine Co., Saskatoon, Canada). The yields of bran obtained from Tx430 Black and Black PI Tall were 16% and 9%, respectively. The bran was separated from the rest of the grain with a KICE grain cleaner (Model 6DT4-1, KICE Industries Inc., Wichita, KS). Sorghum bran was milled in a pill mill (Type 250 CW, Alpine Mill, Augsburg, Germany) to reduce the particle size.

Synthetic colorant standards

The commercial colorant used was FD&C Red No. 3 (Red #3) and FD&C Red No.40 (Red #40) powder. They were kindly provided by Sensient Technologies Corporation (Milwaukee, WI).

Sorghum bran characterization

Color determination

Color parameters of lightness, chroma, and hue of the sorghum brans were obtained using a Minolta CR-310 Colorimeter (Osaka, Japan)

Extraction method

Sorghum brans (0.15 g) were extracted in 25 mL 0.5% citric acid in 70% aqueous ethanol (v/v) for two hours Methanol and shaking at low speed using an Eberbach shaker (Eberbach Corp., MI) for 2 hours. Samples were centrifuged at 2790g for 15 minutes in a Sorvall SS-34 centrifuge (DuPont Instruments, Wilmington, DE) and supernatant was used for total phenol and antioxidant activity. Extracted samples were kept at -20ºC to prevent oxidation.

Total phenol content

Phenol content was measured using the Folin-Cicalteu method as described by Dykes et al (2005). A portion of extracted sample (0.1 mL) was diluted with 1.1 mL of water and then reacted with 0.4 mL of Folin reagent and 0.9 mL of 0.5 M ethanolamine. After 20 min at room temperature, absorbance was measured at 600 nm. Results were expressed as Gallic acid equivalents.

Tannin content

Condensed tannins were measured using the modified vanillin/HCl assay. A stock solution of vanillin reagent was made by mixing a solution 1 gram of vanillin in 100 mL of methanol with a solution of 8% HCl in Methanol. For each sample (0.1500 – 0.3000 g per sample), 8 mL of 1% HCl in Methanol were added and shacked for 10 sec every 10 min for 20 min in a water bath at 30ºC. Samples were centrifuged for 15 min. Two 1 aliquots per sample (1 mL) were placed into two test tubes. The first one was used as a sample and the second one as a blank. For the sample, 5 mL of stock solution of vanillin was added at

15 sec interval to the first sample tube in each pair. As a blank, a 5 mL of a solution made of 4% HCl in Methanol was added every 15 sec to the second sample tube of each pair. Absorbance of each sample was measured at 500 nm after 20 min in the water bath at 30ºC. Difference between the blank and the sample was used for calculations and results were expressed in catechin equivalent per gram.

Methods

Extraction

Each bran (0.5 g) was extracted with 25 mL of 0.5% citric acid in 70% aqueous ethanol in 50 mL centrifuge tubes. The samples were shaken for 2 h at low speed using an Eberbach shaker (Eberbach Corp., MI) and then centrifuged at 2790g for 15 min in a Sorvall SS-34 centrifuge (DuPont Instruments, Wilmington, DE). The supernatant (20 mL) was recovered and evaporated to dryness at 25°C under vacuum in a SpeedVac SC201A (Thermo, Marietta, OH). The dry extracts were stored in the dark at -8°C until analyses.

Sample preparation

Before stability measurements, dry extracts from both black sorghums were reconstituted in 10 mL of 70% ethanol in water. From this reconstituted extracts, an aliquot was taken and dissolved with 70% aqueous ethanol to obtain a concentration of 0.5 mg/mL, with exception of concentration stability samples where different concentrations were prepared. For temperature, a water activity and concentration study, the pH was adjusted to 2 using 0.01 N HCl or 0.01 N NaOH.

The standards FD&C Red No.3 and FD&C Red No. 40 (0.0100g) were dissolved in 10 mL of 70% aqueous ethanol. The pH was adjusted to 2 (except for the pH stability study).

Samples were kept at 25℃ in the dark and they were allowed to equilibrate for 2 hours before the first measurement. Measurements were performed at 0 h, 1 day, and every 7 days for 13 weeks. Analyses were done in triplicates

pH stability

Aqueous ethanol acidified solutions of black sorghum brans, FD&C Red No.3 and FD&C Red No. 40 ranging from pH 1 to pH 11 were prepared using 0.01 N HCl or 0.01 N NaOH.

Temperature stability

Samples with a concentration of 0.5 mg/mL and pH 2 were kept in the dark at different temperatures: -8℃, 4℃, 25℃ and 50℃. Samples were allowed to equilibrate for 2 hours before the first measurement. Measurements were performed at 0 h, 1 day, and 7 days for 13 weeks. Analyses were done in triplicates.

Water activity stability

Samples were adjusted to a concentration of 0.5 mg/mL using glycerol to obtain water activity of 0.20; sucrose to obtain water activity of 0.85, 0.95, and water to obtain a water activity of 1.00.

FD&C Red No.3 and FD&C Red No. 40 were dissolved in the different solvents used for the sorghum brans.

Concentration stability

Tx430 Black bran extract (BS) was adjusted to a concentration of 0.5, 1.0, 5.0, 10, 20, and 40 mg/mL. Black PI Tall bran extract (BTS) was adjusted to a concentration of 0.1, 0.5, 1.0, 5.0, 10, and 20 mg/mL.

Color measurements

CIELAB parameters were determined using the D-65 diffused illumination of a Minolta CT-310 colorimeter (Konica Minolta Inc.; Mahwah, NJ). The parameters measured were L (lightness), a* (redness), and b* (yellowness) and also measured and reported as L (lightness), C (chroma), h (hue angle) because the common La*b* coordinates are difficult to interpret independently.

Spectrophotometer measurements for thermal stability

BS and BTS were diluted to obtain a maximum absorbance value of 0.80±0.01, and were scanned at wavelengths from 390 to 700 nm using a Hewlett Packard 8452A photodiode array spectrophotometer (Agilent Technologies; Palo Alto, CA). The maximum absorbance for pH 1 to 11 was obtained.

Thermal stability was done using a thermoblock (Fisher Scientific; Pittsburg, PA). Samples were placed in 5.0 mL cryogenic vials and immersed in the Isotemp 1016S circulation water bath at 99°C (±1°C) for 0, 30, 60, 90, and 120 min. Samples were read using the spectrophotometer at their maximum wavelengths of absorbance using semimicro-cuvettes. Analyses were done in triplicates.

Statistical analysis

Analysis of variance (ANOVA) was performed using SPSS v11.5 for Windows (SPSS Inc.). Differences between means of the three replicates were analyzed with Tukey HSD test using a confidence level of 95%.

CHAPTER IV

SORGHUM BRAN CHARACTERIZATION

Color characteristics of sorghum brans

Lowest L-value was obtained by Tx430 Black sorghum bran (44.24) meaning that this bran had the darkest color (table IV).These results were expected because L-values decreased with increasing black pigmentation of the pericarp (Gous, 1989). Chroma values of both sorghum brans were low and similar, which means low color intensity. The highest hue was obtained by PI Tall Black (43.11) meaning that the bran is more in the orange region. TX430 Black had hue values of 31.91, meaning that the bran is closest to the red region.

Table IV. Color parameters of sorghum bran Tx430 Black and Black PI Tall

Sorghum bran	Lightness	Chroma	Hue
Tx430 Black	44.25 ± 0.05	9.16 ± 0.05	31.91 ± 0.08
Black PI Tall	49.12 ± 0.02	9.76 ± 0.08	43.11 ± 0.09

Total phenol content

Phenolic compounds of cereal grains are mainly found in the pericarp layers of the grains so the phenolic compounds are concentrated in the bran. Black PI Tall sorghum bran had higher levels of total phenols (55.99 mg GAE/g) than Tx430 Black sorghum bran (Table V). These results are confirmed by Gous (1989) who showed that increasing black pigmentation of the pericarp is associated with a decrease in the amount of total phenolic compounds.

Table V. Phenol and tannin content of Tx430 Black and Black PI Tall sorghum bran extracted with 0.05% citric acid in 70% aqueous ethanol

Sample	Phenol content mg GAE/g	Tannin content mg CE/g
Tx430 Black	17.72 ± 0.67	-
Black PI Tall	55.99 ± 1.29	30.06 ± 0.88

Tannin content

Tx430 Black sorghum bran does not contain pigmented testa so did not had any significant quantities of tannins (Table V). PI Tall Black sorghum bran had high levels of tannins (30.06 mg CE/g). The testa layer is located below the endocarp surrounding the endosperm so most of the sorghum tannins were contained in the bran (Awika, 2000). That is the reason that Black PI Tall bran showed high levels of tannin content than Black PI Tall sorghum grain which had levels of condensed tannins between 11.9 and 12.0 mg CE/g (Dykes et al., 2005). Many authors have used different solvents for tannin extraction; therefore, the comparison of tannins levels is difficult among the different studies.

3-deoxyanthocyanins profile of black sorghum brans

Black PI Tall is a black sorghum which contains tannins. On the other hand, Tx430 Black is also a black sorghum without tannins. The main 3-deoxyanthocyanins detected in the varieties of Tx430 Black and Black PI Tall sorghum bran, when they were extracted with citric acid in aqueous ethanol, were luteolinidin, apigeninidin, 5-methoxyluteolinidin, and 7-methoxyapigeninidin (Figs. 7 and 8). These compounds were identified using commercial standards. These 3-deoxyanthocyanins are structurally different from the rest of the anthocyanins. Apigeninidin had maxima absorption at 468 nm and luteolinidin at 482 nm luteolinidin. In a pH 1 solution these compounds appear orange (luteolinidin) and yellow (apigeninidin). On the other hand, the common anthocyanins have a red color (Winefield et al, 2005; Awika, 2003).

Tx430 Black sorghum extracted with 0.5%citric acid in 70%aqueos ethanol had an average of 1134 µg/g of 3-deoxyanthocyanins. The profile showed that luteolinidin and apigeninidin are in equal proportions (415.5 and 475.8 µg/g, respectively) followed by 5-methoxyluteolindin with 237.9 µg/g and 7-metoxyapigeninidin with 215.8 µg/g (Njongmeta Nenge, L.A. et al. 2007)

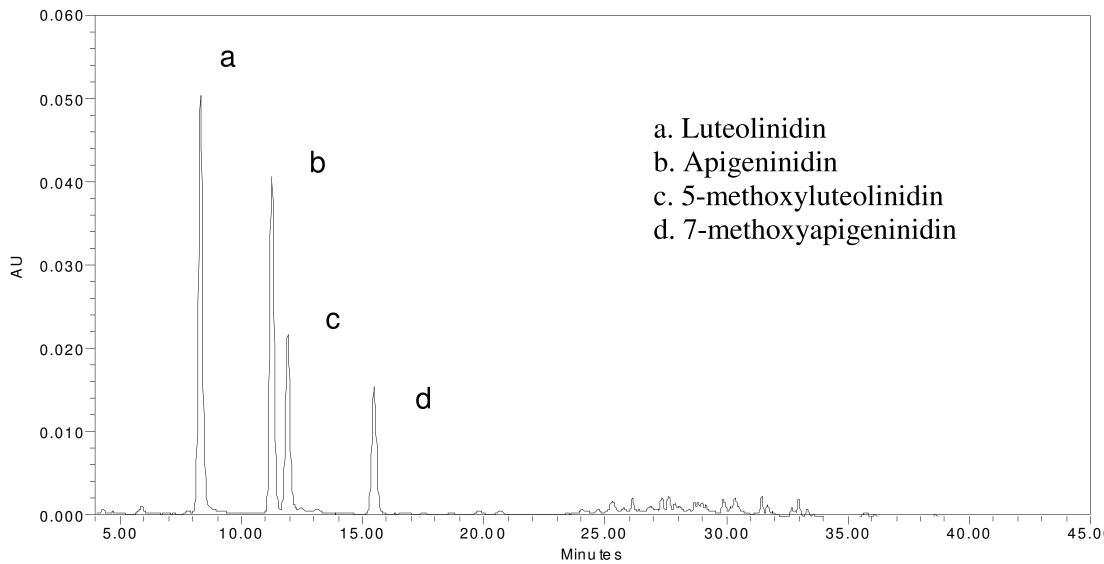


Fig. 7. HPLC chromatograms of 3-deoxyanthocyanins of Tx430 Black sorghum bran (BS) extracted with 0.5% citric acid in aqueous ethanol detected at 485 nm. *Source:* Njongmeta, LA. 2008 CQL, Texas A&M.

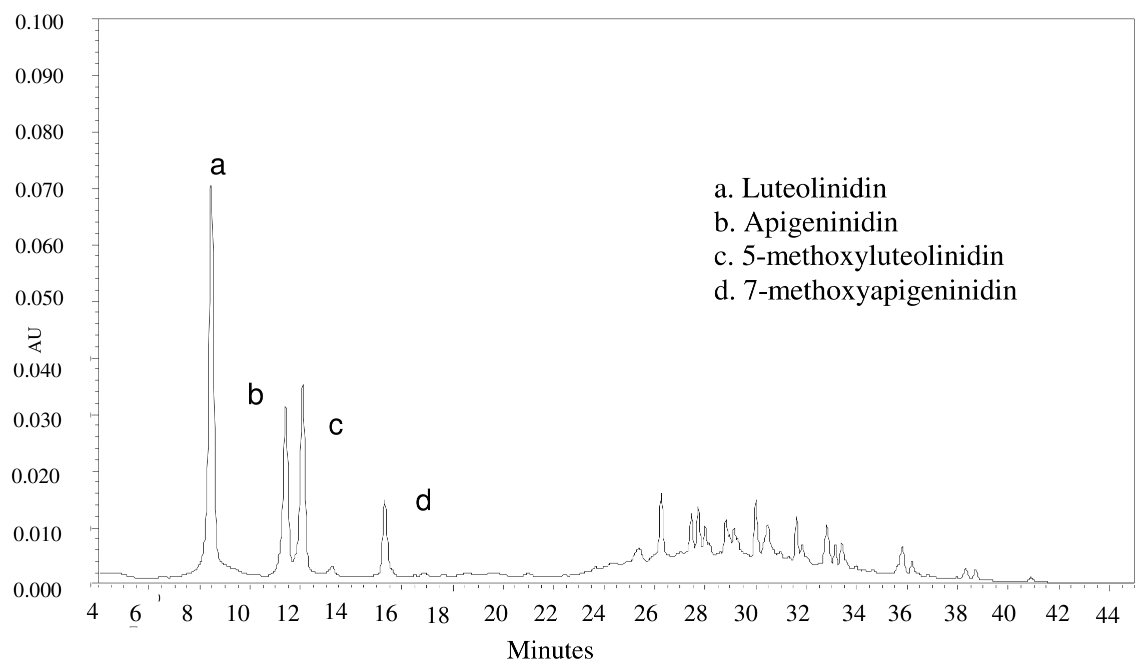


Fig. 8. HPLC chromatograms of 3-deoxyanthocyanin of Black PI Tall sorghum bran (BTS) extracted with 0.5% citric acid in aqueous ethanol detected at 485 nm. *Source:* Njongmeta, LA. 2008 CQL, Texas A&M.

CHAPTER V

THERMAL STABILITY TEST

Spectral characteristics of Tx430 Black and Black PI Tall sorghum bran

pH is considered one of the factors that mostly affects the stability of anthocyanins. This factor limits the use of the anthocyanins of fruit and vegetable in many food products. It is well known that 3-deoxyanthocyanins from sorghum have good stability even at higher pH solutions (Dykes and Rooney 2006**;** Awika et al., 2004a; Gous, 1989). Before doing experiments on long-term stability of the Tx430 Black and Black PI Tall sorghum brans, short-term stability experiments were performed.

The visible λ_{max} of Tx430 Black sorghum bran extracts (BS) was the same at 492 nm for all pHs (Fig. 9). When pH increased from pH 1 to 6 the absorbance readings decreased. The highest absorbance was observed in sorghums at pH 1 (0.463) and 2 (0.442). pH 8, 9, 10, and, 11 have similar absorbance (0.240-0.275).

According to Hendry & Houghton (1992), when compounds absorb light at 490 nm the color perceived is yellow, and at 525-540 nm the color perceived is orange. Awika (2003) mentioned that the 3-deoxyanthocyanidins in methanol appeared yellowish orange for apigeninidin and reddish orange for luteolinidin. As the main 3-deoxyanthocyanidins present in the sorghum bran are apigeninidin and luteolinidin, the color is yellow-orange.

For Black PI Tall sorghum bran extracts (BTS) the maxima absorbance for pH from 1 to 9 was 496 nm (Fig. 10). Sorghum bran at pH 1 presented the highest absorbance (0.873) followed by sorghum bran at pH 2 (0.596). The maximum absorbance for pH 10 was at 510 nm and for pH 11 at 560 nm. The color perceived at these pHs were more in the orange and orange-red color, respectively.

In a study conducted by Cevallos-Casals and Cisneros-Zevallos (2004), the behavior of the anthocyanins was different than presented in this study. They observed a bathocromic shifts (an increase of λmax to higher wavelengths) in red sweet potato, purple corn, purple carrot, and red grape colorants at pH above 5. They also observed a decrease in the absorbance (hypochromic shift) of purple corn and commercial red grape extracts at pH from 4 to 6. These extracts are rich in non-acylated anthocyanins, which showed colorless structures at these pHs. In another study, change in color was observed in a cranberry cocktail at different pHs (Cevallos-Casals and Cisneros-Zevallos, 2004). Anthocyanins show the highest absorbance at pH 1; at pH 4.5 the absorbance was almost zero, being nearly colorless (Fennema, 1996). The changes in the absorption spectra are due to shifts in the equilibrium of the four anthocyanins species: the flavylium cation, the quinonoidal base, the carbinol pseudobase and the chalcone (Reyes Mora, 2002).

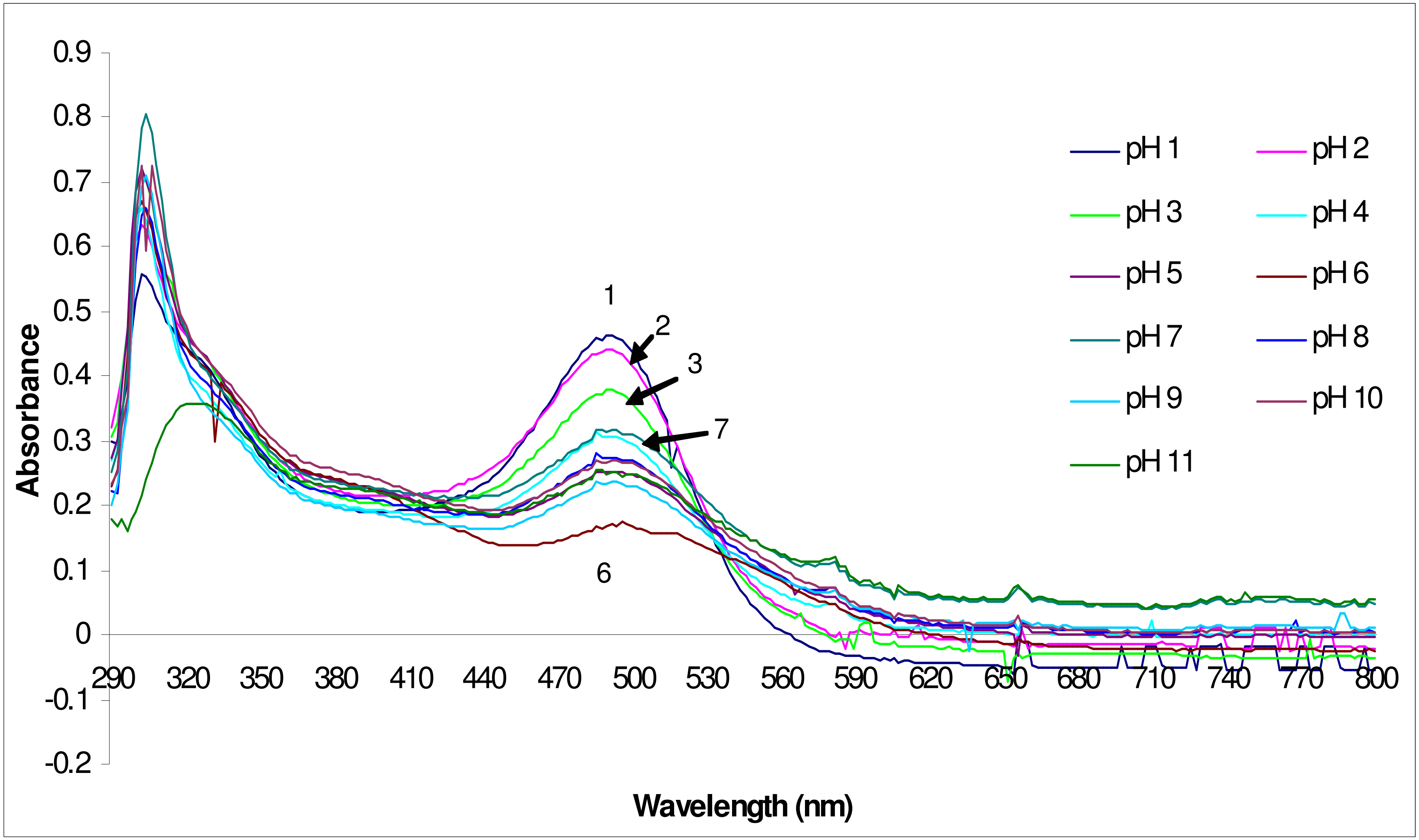


Fig. 9. Spectrum charts for Tx430 Black sorghum bran (BS) at different pHs, after 2 hours at concentration of 5mg/mL of 0.5% citric acid in 70% aqueous ethanol protected from light.

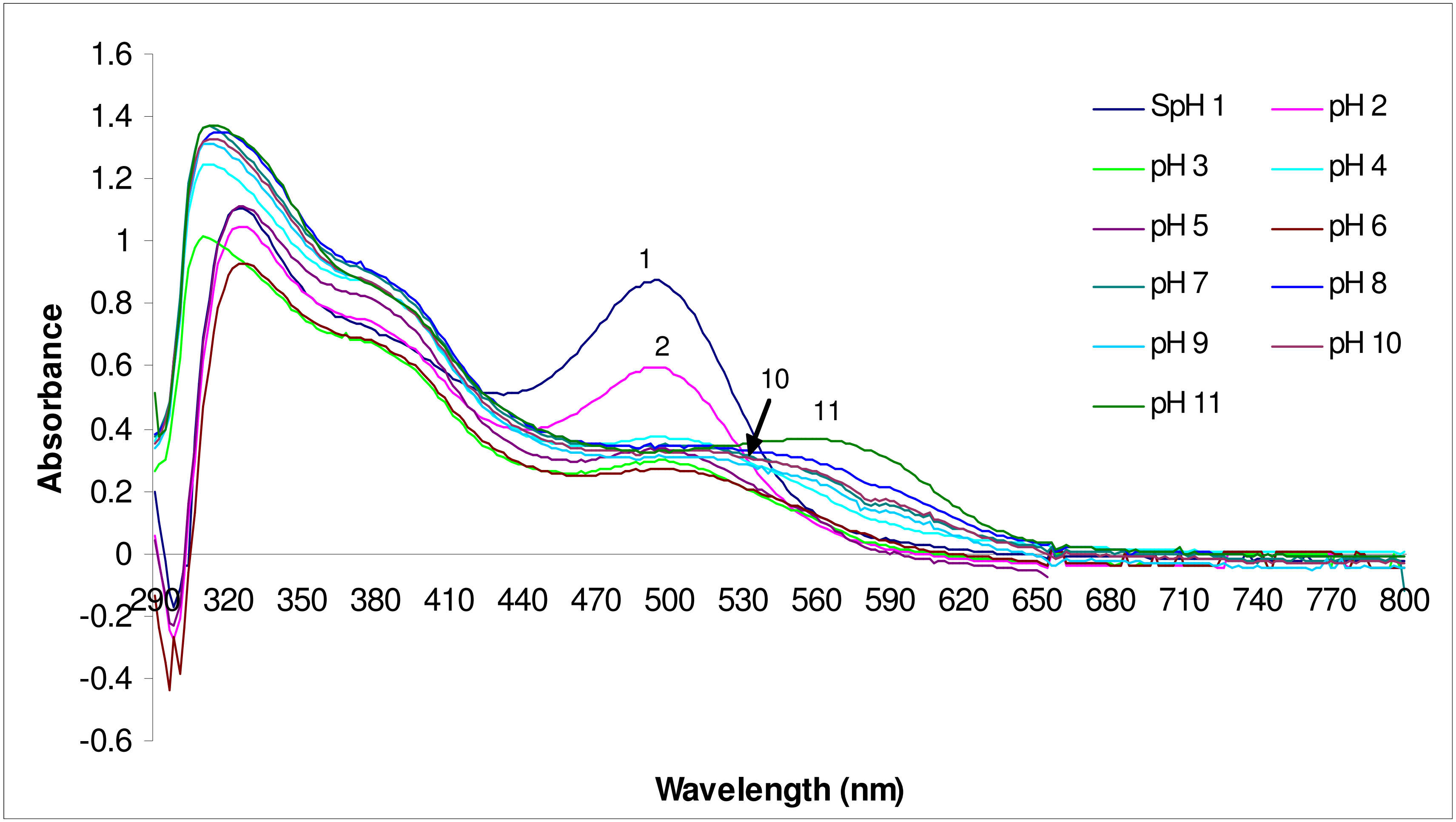


Fig. 10. Spectrum charts for Black PI Tall sorghum bran (BTS) at different pHs, after 2 hours at concentration of 5mg/mL of 0.5% citric acid in 70% aqueous ethanol protected from light.

Unlike common anthocyanins found in fruits and vegetables, which show virtually no absorbance at ph 4 -5 (Awika at el., 2004b), black sorghum bran showed absorbance, at all pH range even at pH neutrality at their maxima wavelength (492 for Tx430 Black variety, 496 for Black PI Tall). This phenomenon is due to the presence of luteolinidin and apigeninidin which had significant absorbance at neutral pH (Awika at el., 2004b). No bathocromic shifts were found at the different pH of both sorghum varieties, except at pH 10 and 11 of Black PI Tall variety.

At low pH, the 3-dexoyanthocyanins from sorghum exist as flavylium cation. As the ph decreases, the red flavylium is hydrated to give the carbinol structure. As the pH of BS increases, the colorless carbinol is formed having which discolors the extracts. At pH 1 and 2 the flavylium cation is present in BTS. At pH 10 and 11 BTS had high absorption due to the presence of the blue quinonoidal structures. These data suggest a potential use of the 3-deoxyanthocyanins from sorghum bran extracts as food colorant due to the stability of absorbance at different pHs.

pH stability of Tx430 Black and Black PI Tall sorghum brans

After two hours of equilibration the absorbance of BS and BTS at pH from 1 to 11 was read. The measurements were done at the maxima wavelength of each pH.

There was not significant decrease in absorbance of the BS from pH 3 to 11 after two hours (Fig. 11). This means the extracts had good stability over time at 98 °C. The absorbance of extracts at pH 1 showed an increase in intensity of more than 70% after 30 minutes; however, the absorbance intensity decreased again and after two hours the absorbance was not significantly different from time zero. The color retention increased by 17% after 2 hours. Extracts at pH 2 also showed an increase in absorbance with color retention of 37% over time.

The absorbance of BTS at pH 3-6 and 8-10 did not change over time. On the other hand, BTS at pH 7 and pH 11 showed similar absorbance values after one hour (Fig. 12). From time zero to 30 minutes, the absorbance of pH 1 and pH 2 extracts increased by 94% and 83%, respectively. This increase may be due to the formation of brownish products from the degradation of anthocyanins. The absorbance of the extracts at pH 3 to 11 was statistically similar ($P<0.05$) over two hours. Extracts at pH 1 and pH 2 showed significantly higher absorbance readings than the rest of the samples, likely due to predominant presence of flavylium cation. The color retention of all pH increased except at pH 3 which decreased by 20%. Extracts at pH 1 and pH 2 had increased color retention of 90 and 104%, respectively.

The behavior of black sorghum extracts was different from pigments from purple corn, sweet potato, purple carrots and grape. With time, the absorbance of the vegetable/fruit anthocyanins at different pH decreased with decreased color retention (Cevallos, 2001). BS and BTS retained a high % of color retention over time, demonstrating good stability. However, the extraction solvent of the black sorghum differed from Cevallos method. In this study the extraction solvent was citric acid in aqueous ethanol and in Cevallos study the extraction solvent was nanopure water.

In general, the absorbance values of the black sorghum bran extracts at short time and high temperature were stable through time at pH 1 to 11. The stability of crude pigment extracts of a black variety of sorghum was relatively good at pH up to 8 compared to common anthocyanins (pelargonidin, cyanidin, peonidin, delphinidin, malvidin, and petunidin) (Gous, 1989).

Black sorghum bran as a potential food colorant

Extraction of anthocyanins with acidified alcohol has been the common method used for sorghum. The classical alcohol used to remove anthocyanins from plant materials is methanol and a small amount of HCl (<1%) (Markakis, 1982; Bridle and Timberlake, 1996). In sorghum, extraction with HCl-methanol was 60% higher than extraction using acetone. This solvent helps to extract the 3-deoxyanthocyanins from sorghum preventing them from forming other compounds (Awika et al. 2004b). Extraction of the pigments of the sorghum Shawaya with acidified methanol was most efficient compared to other solvents. When this variety was extracted with acidified ethanol, only about 80% of the pigments where extracted compared to acidified methanol, but acidified ethanol has the advantage that it is non-toxic (Gous, 1989).

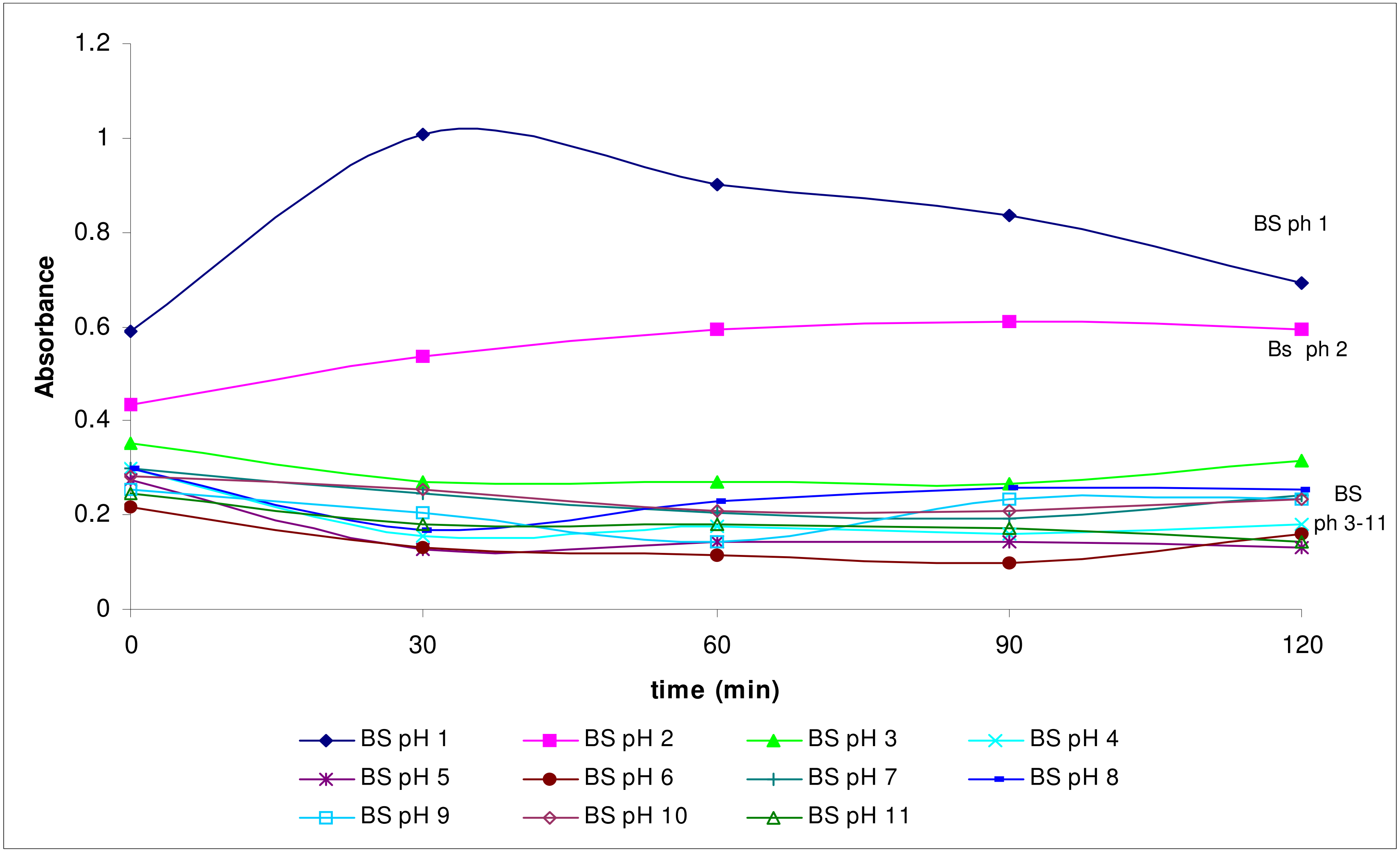


Fig. 11. Accelerated absorbance stability of Tx430 Black sorghum bran (BS) at 98°C for two hours at concentration of 5mg/mL of 0.5% citric acid in 70% aqueous ethanol protected from light.

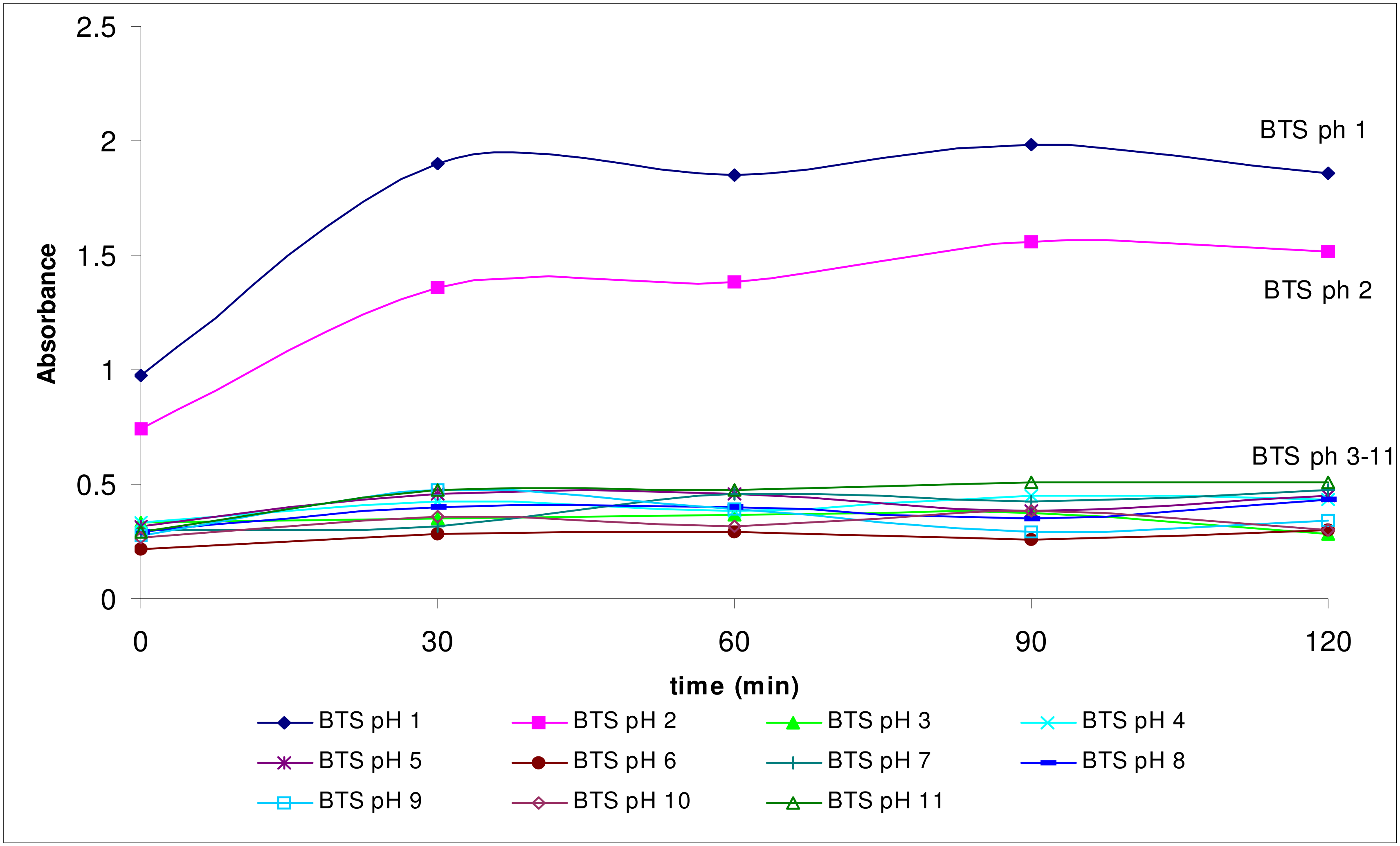


Fig. 12. Accelerated absorbance stability of Black PI Tall sorghum bran (BTS) at 98°C for two hours at concentration of 5mg/mL of 0.5% citric acid in 70% aqueous ethanol protected from light.

In a study performed with cherry plum, the anthocyanins from the leaves were extracted using an ethanol-HCl mixture, which obtained better results than using the common extraction method (Markakis, 1982). Citric acid instead of HCl has been used achieving good extractions. Citric acid is less corrosive than HCl, chelates metals and maintain the low pH (Bridle and Timberlake, 1996). The solvent used to extract the 3-deoxyanthocyanins from the black sorghum bran was citric acid in aqueous ethanol (Njongmeta Nenge, L.A. et al. 2007). The solids extraction (around 32%) was lower than the common method used but this solvent was chosen because ethanol is a solvent used in the food industry and citric acid in small amounts can be consumed by humans without damage to their health.

The use of anthocyanins and other natural pigments as possible food colorants has been pursued because most synthetic colorants are not believed safe and effective. Economic, technical and legal considerations determine the choice of the raw plant materials used in commercial food products. The plant material should be available in large quantities, the price should be reasonable, the process to extract and obtain the pigment should be easy, and most important the final product must appear good to the consumer and must meet the legal requirements of the government (Markakis, 1982).

Black sorghum bran could be a good source of natural food colorant (Awika, 2003). This crop is largely available and the cost to produce, store and extract is not expensive compared to other natural sources of food colorant like strawberries, grapes, and red cabbage.

In conclusion, at different pHs, the black sorghum bran extracts showed good stability at 98℃ for 2 hours. This demonstrates a good potential of the black sorghum extracts to withstand thermal processing than fruits and vegetables extracts. Further experiments are needed to determine the stability of 3-deoxyanthocyanins in different conditions which mimic food systems.

CHAPTER VI

LONG-TERM TESTS

pH stability

pH has a marked influence on the anthocyanin color in aqueous media. Anthocyanins are known to display huge color variations in the pH range form 1 to 14. But, pH affects both shade and color intensity. (Hendry and Houghton, 1992). The intensity decreases as the pH increases. The stability of the anthocyanins is also influenced by pH.

In acidic or neutral media, four anthocyanin structures exist in equilibrium: the flavylium cation AH^+, the quinonoidal base A, the carbinol pseudobase B, and the chalcone C. Below pH 2, the anthocyanins exists primarily in the form of the flavylium cation. When the pH increases, a red or blue quinonoidal form occurs as a result of a rapid proton loss. This quinonoidal form usually exists as a mixture. Then, a hydration of the flavylium cation gives a colorless carbinol or pseudobase. This reaction in most anthocyanins occurs at pH values from 3 to 6. Further increase in pH resulted in the formation of colored quinonoidal bases as well as yellow chalcone species (Mazza and Miniati, 1993). This means that at pH below 2 the anthocyanin solutions show their highest red or yellow coloration. As the pH of the solution increases their color fades, and at pH 4 to 6 most anthocyanins are colorless. Further increases in pH give solutions that are purple and blue and with heat treatment may change to yellow. Acidification is used to reverse this process (Timberlake and Bridle, 1980).

The pH affects the stability of the anthocyanins enormously. The unique 3-deoxyanthocyanins present in sorghum are more stable than common anthocyanins even at neutral and higher pHs, where the 3-deoxyanthocyanins show color. This phenomenon may be because the quinoidal bases dominate above pH 3 as these compounds have reduced tendency to form the colorless pseudobase (Clifford, 2000).

To see the behavior of the color changes of the 3-deoxyantocyanins in BS and BTS, experiments at different pH were performed over 13 weeks to monitor the changes in the visual color in terms of lightness, chroma, and hue values.

Changes in visual color attributes of Tx430 Black sorghum bran extracts at different pHs

The L-values of the BS and the standards Red #3 and Red #40 measured during 13 weeks are in Fig. 13. Except for Red No. 3 at pH 2, all samples were in the range of lightness between 45 and 75. The brightness of BS at pH 8 to 11 increased over the first 7 weeks; L-values remained constant after week 7. Values were higher at pH 8-11 (between 70 and 75) than samples at pH 1 to 7, implying that at high pH BS is brighter than at low pH. L-values of Red #3 at pH 5 and pH 11 were similar to the L- values of sorghum extracts.

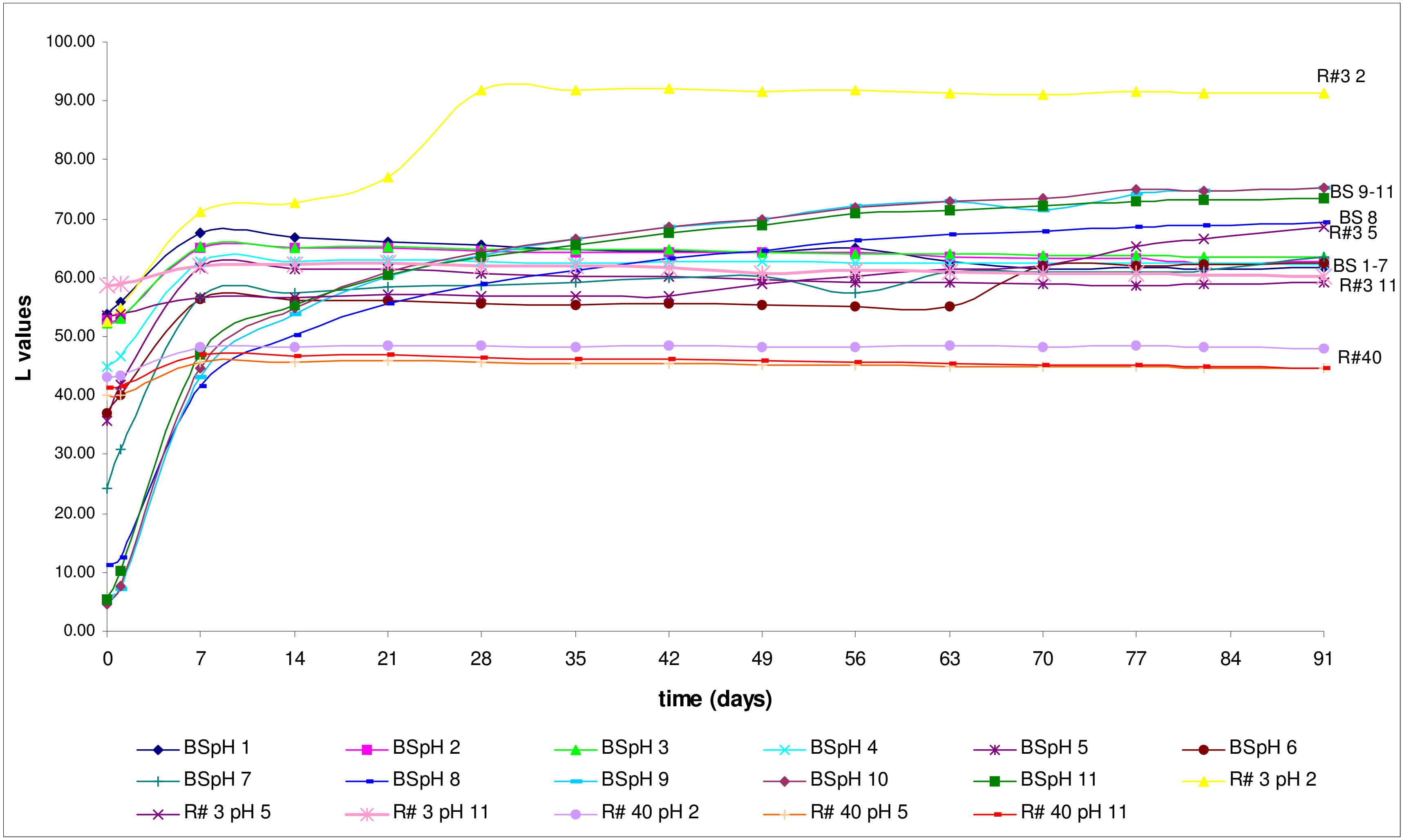


Fig. 13. Lightness values of Tx430 Black sorghum bran extracts (BS) and standard Red No.3 (R# 3) and Red No.40 (R#40) over time at different pH. Concentration of 5mg of extract/mL of citric acid in aqueous ethanol at 25℃ kept in the dark.

Red #3 at pH 2 was the brightest (91 after 4 weeks) of all the samples. Contrary to this, Red #40 at all pHs (2, 5, and 11) were the least bright with values between 45 and 50. The curves of Red #40 were stable over time. This means that lightness of synthetic colors is affected by pH.

The chroma values of the BS were constant over the 13 weeks (Fig. 14). After 1 week, BS at pH 1, 2, and 3 were constant and the high C-values means that those sorghum samples were the most intense. Intensity of color is an important attribute that makes food more appealing to consumers. The chroma level of the acidified extracts was compared with the chroma level of Red #40 at pH 2, 5, and 11. The acidified solutions of BS may be a good substitute (in terms of vividness) for Red #40 in food products because the C-values were not significantly different between them. For BS at pH 6 to 11, the C-values were not significantly different between samples; the values were constant (62-65). The synthetic colorants were not close to the range of the C-values of sorghum extracts. Red #3 at pH 5 and pH 11 had the highest chroma values (> 120) indicating they were the most vivid solutions. These values were close to the C-values of the BTS at pH 1, 2, 3 and Red #40. The dullest sample was Red #3 at pH 2, which had low C values (below 40). This low value is because the stability of the Red #3 is poor under acid conditions (Francis,1999).

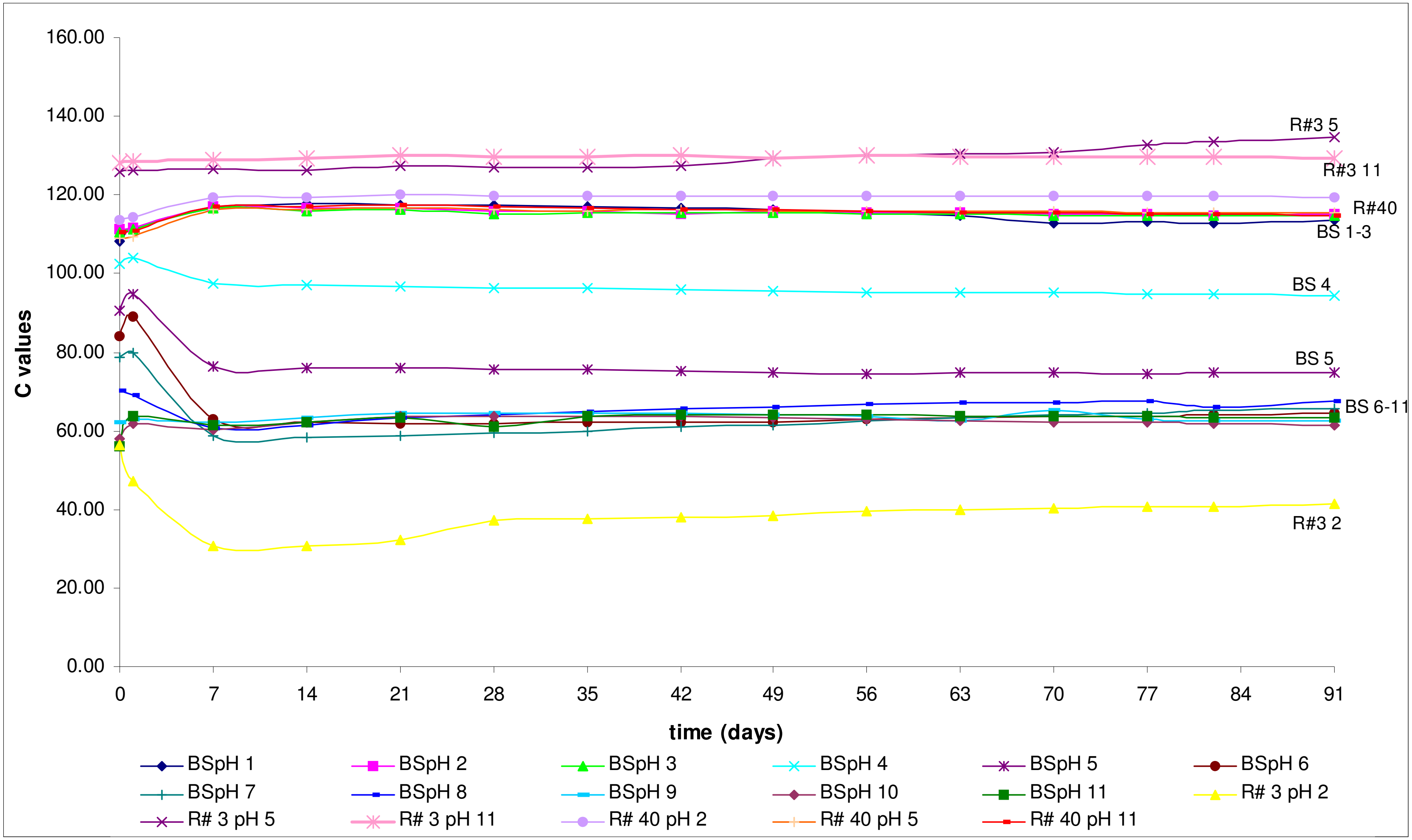


Fig. 14. Chroma values of Tx430 Black sorghum bran extracts (BS) and standard Red No.3 (R# 3) and Red No.40 (R#40) over time at different pH. Concentration of 5mg of extract/mL of citric acid in aqueous ethanol at 25°C kept in the dark.

Hue is referred to as the actual color; values between 0° and 90° means that the color is orange. If the hue value increases the color is more yellow. On the other hand, if the hue value decreases the color is more red. All samples started with low values of hue (red range) (Fig. 15). After one week, all the samples increased in hue values. The color was red and orange and then increased to the yellow range. BS at pH 1, 2, and 3 had constant hues of 63-65 after 3 weeks. These angles represent the orange color closer to the yellow range than the red range. BS at pH 4, 5, 6, and 7 had hue values between 53 and 57. Over time the hue values increased. These extracts were more red-orange than the extracts at pH 1, 2, and 3.

The hue values of BS at pH 8, 9, 10, and 11 were the highest with no significant differences among them. Statistically ($P<0.05$), these samples had the most significant change in hue values over time. On day 0, these samples were the most red with values around 10, then with time, the hue values increased, and after 7 weeks the values were above 70. By the end of the experiment, these samples showed an orange-yellow color. This behavior may be due to gradual conversion of the reddish quinoidal bases to colorless chalcones or pseudobases of the 3-deoxyanthocyanins over time. Red #3 at pH 2 was in the range of orange-yellow color showing similarity with the BS in high pHs. Alkaline BS can replace Red #3 in food products (e.g. vegetable juices). The hue values of Red #3 at pH 5 and 11 were slightly lower than the BS at neutral pH, therefore BS could replace Red #3 at these conditions. Red #40 at the different pHs had the lowest h-values (around 42) with an orange color.

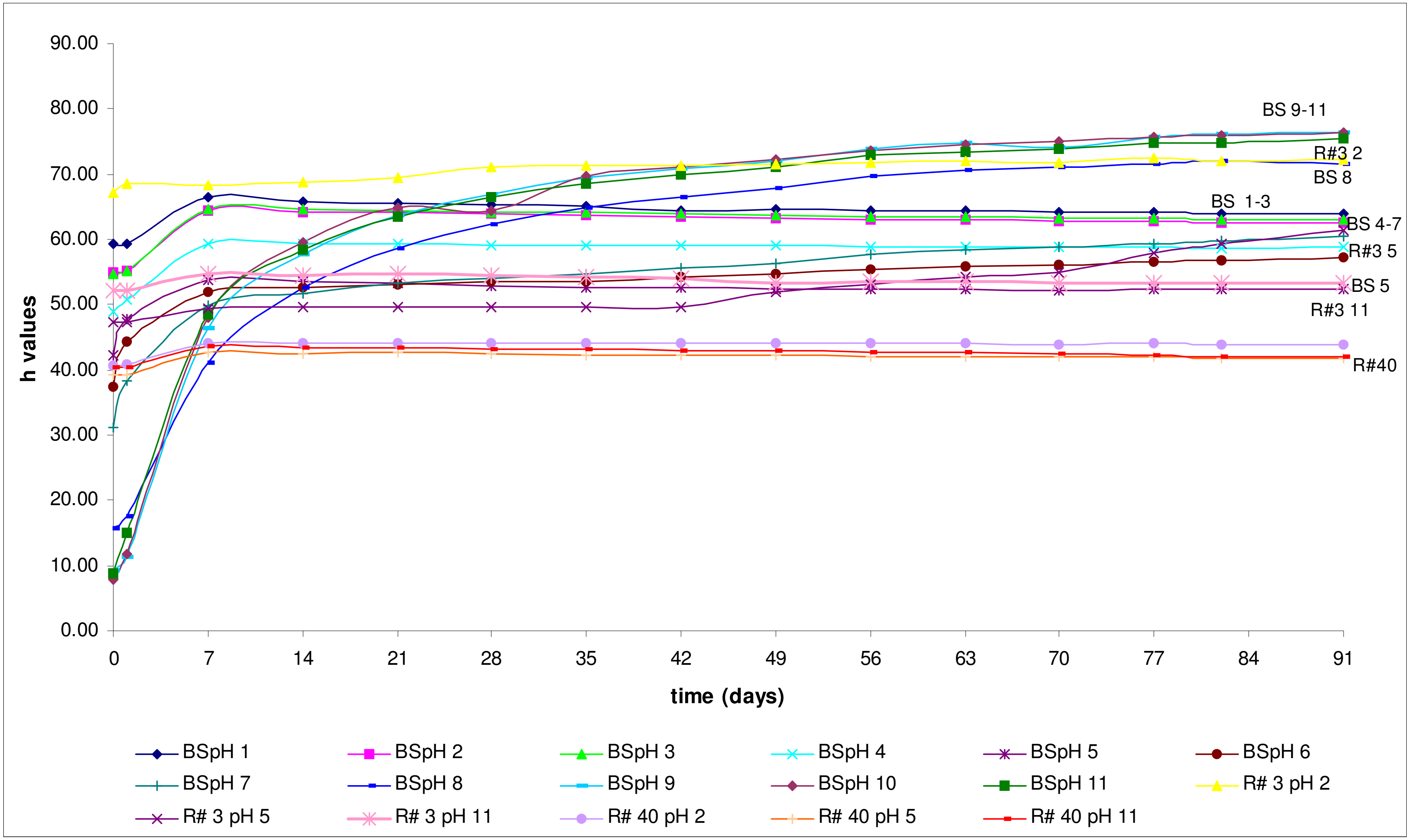


Fig. 15. Hue values of Tx430 Black sorghum bran extracts (BS) and standard Red No.3 (R# 3) and Red No.40 (R#40) over time at different pH. Concentration of 5mg of extract/mL of citric acid in aqueous ethanol at 25℃ kept in the dark.

Changes in visual color attributes of Black PI Tall sorghum bran extracts at different pHs

L-values of the variety of BTS at all pH studied were very stable (Fig. 16). The lightness of all sorghum bran samples at pH 1 and 2 were stable after 7 weeks, decreasing the L-values between 30 and 35. After 1 week, BTS at pH 3 to 11 had stable L-values between 30 and 35 during 13 weeks. The sorghum extracts did not match the lightness of the synthetic colorants. Red #3 at pH 2 had the highest L-values from 50 to 90 after 4 weeks. L-values of Red #3 at pH 5 were statistically different ($P<0.05$), and the L-values of Red #3 at pH 11 were constant around 60. The lightness of Red #40 at pH 2, 5, and 11 was in between the Red #3 and the BTS values between 44 and 50.

The vividness of BTS at pH 1 and pH 2 were not significantly different after 2 weeks and BTS at pH 3 to 11 were stable over time (Fig. 17). Chroma of all BTS was stable (87-94). Red #3 at pH 2 produced the dullest color of all the samples. This is due to the lack of stability in acidified solutions. Red #3 at pH 5 and pH 11 was the samples that showed the most vivid colors. Followed by Red #40 which had C-values around 120. The BTS did not have C-values that were close to those of the standards, meaning that at these conditions BTS might not replace the synthetic colorants.

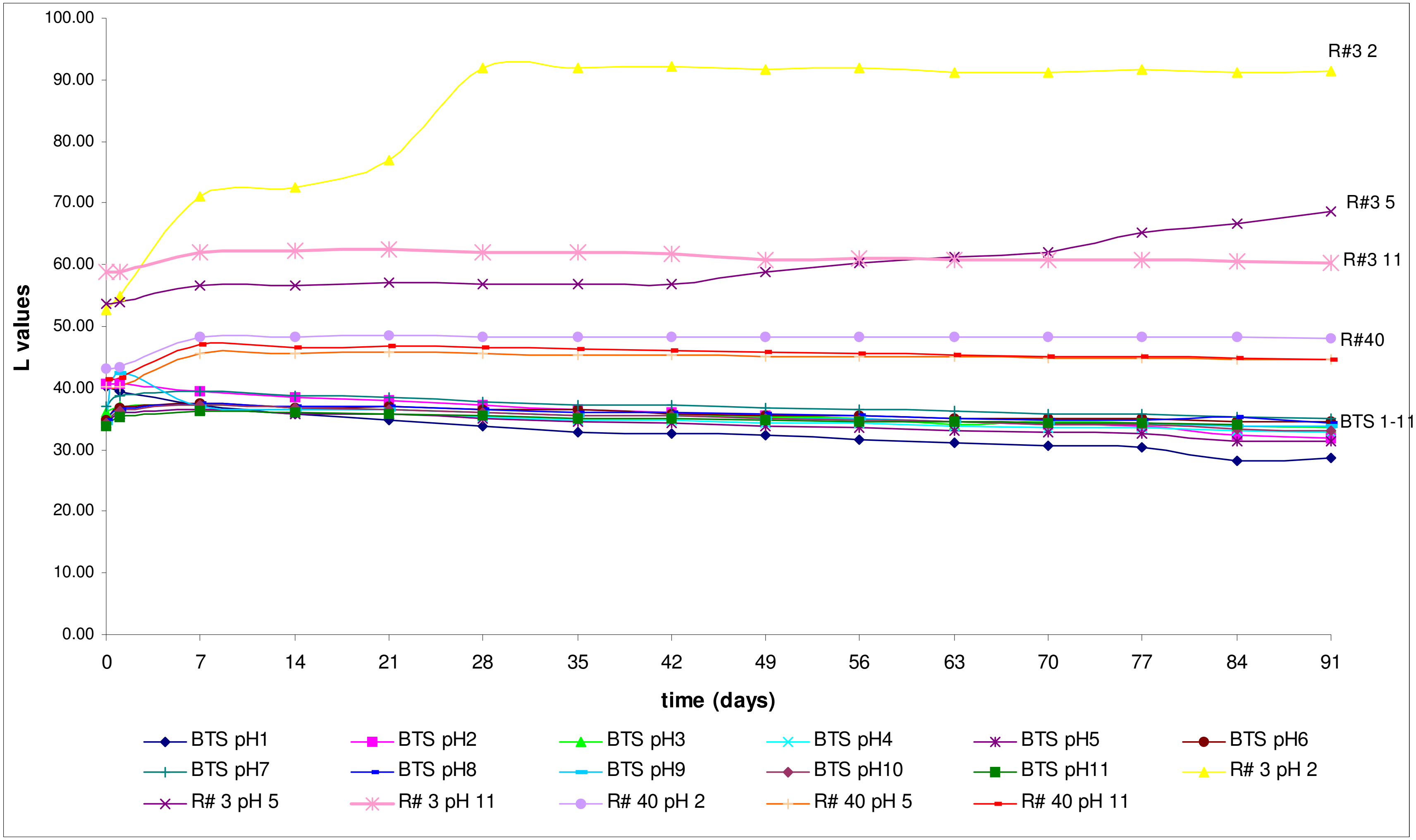


Fig. 16. Lightness values of Black PI Tall sorghum bran extracts (BTS) and standard Red No.3 (R# 3) and Red No.40 (R#40) over time at different pH. Concentration of 5mg of extract/mL of citric acid in aqueous ethanol at 25°C kept in the dark.

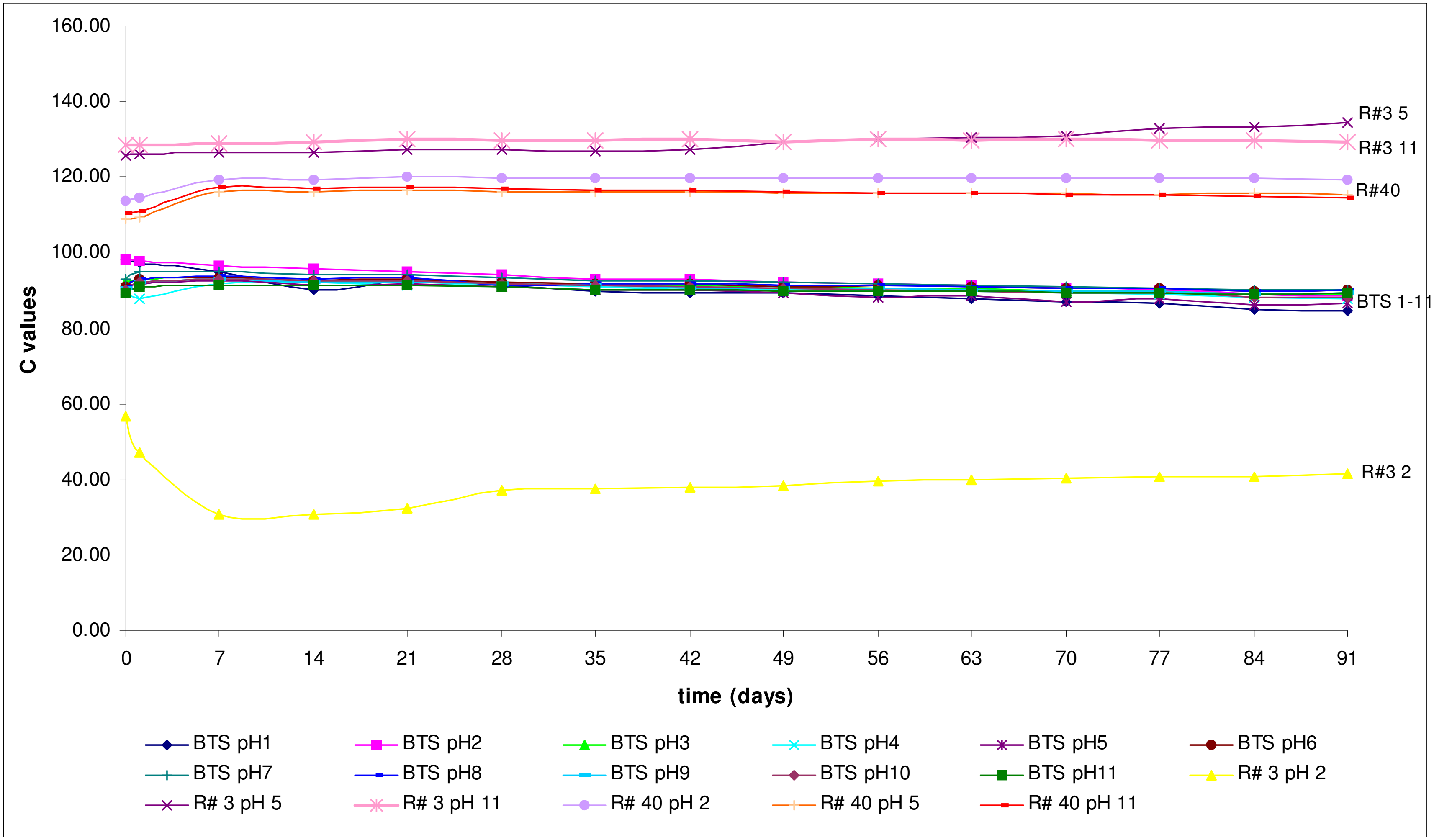


Fig. 17. Chroma values of Black PI Tall sorghum bran extracts (BTS) and standard Red No.3 (R# 3) and Red No.40 (R#40) over time at different pH. Concentration of 5mg of extract/mL of citric acid in aqueous ethanol at 25℃ kept in the dark.

The hue values of the sorghum samples were between 35 and 44, after 1 week (Fig. 18); those values correspond to orange color. BTS at pH 1 started with higher hue values (close to yellow-orange color) than the rest of the BTS and then decreased towards red color. Hue values of the BTS at pH 2 were stable after 2 weeks. BTS at pH 3-11 were not significant different over time. Red #3 had highest hue at all pH. Red No. 40 at the three pHs measured was not significantly different over time; these values were similar to BTS (between 40 and 44). These results indicated that BTS at all pHs can be a good substitute for Red #40.

As potential colorants, common anthocyanins are compared with some other natural red colorants like betalains and with red synthetic colorants, but they are only colored at low pH (Timberlake and Bridle, 1980: Cai and Corke, 1999) and their stability at neutral and alkaline pH solutions is not good (Rein, 2005). Our data show that the 3-deoxyanthocyanins from the BS and BTS are very stable under different pH conditions and may be good substitutes for the red synthetic colorants.

Anthocyanins from fruits and vegetables produce different colors from acidic to alkaline pH; intensity of color is also changed, becoming dull at higher pH (Rein, 2005). In contrast to those studies, the 3-deoxyanthocyanins have stable color at the different pH probably due to copigmentation effect as the result of flavonoids-anthocyanidin condensation reactions (Gous, 1989). In a study with red sweet potato and purple corn (Cevallos-Casals and Cisneros-Zevallos, 2004), radish, and potato extracts (Giusti and Wrolstad, 2003), the hue,

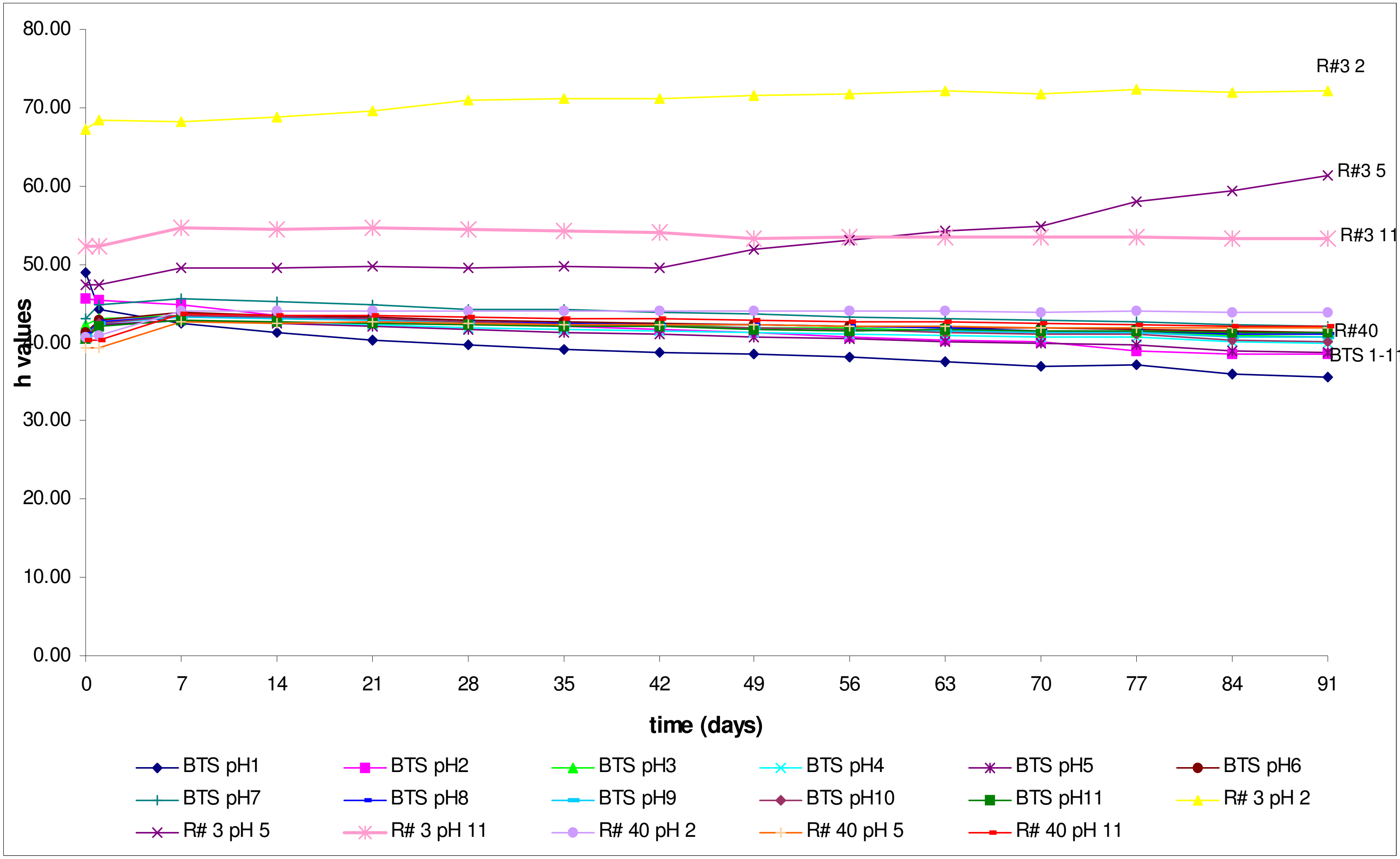


Fig. 18. Hue values of Black PI Tall sorghum bran extracts(BTS) and standard Red No.3 (R#3) and Red No.40 (R#40) over time at different pH. Concentration of 5mg of extract/mL of citric acid in aqueous ethanol at 25℃ kept in the dark.

chroma and lightness change as the pH change. Red sweet potato was a pink-red color at pHs up to 3. An increase in temperature at pHs up to 8 changed the color to blue hues while at higher pHs, the color was changed to yellow. At low pH, purple corn and purple carrot had an orange-red color and an increase in temperature changed the color to light pink which shifted to brown (Cevallos, 2001).

To make a better comparison between the two varieties of black sorghum bran extracts and the synthetic colorants, the hue and chroma were plotted. The changes in color of the samples at different pHs at 1 and 13 week are shown in Figs. 19, 20, 21, 22, and 23. At low pH (Fig. 19), the hue values of BTS were comparable to Red #40 while those of BS were comparable to Red #3. The chroma values were different. At neutral and alkaline pH (Figs. 20 and 21), the behavior of the sorghum bran extracts was similar. BTS were comparable in hue and chroma to Red #40. BS was comparable to the hue values of Red #3 under certain conditions. At neutral pH Red #3 changed color over time and at high pH BS changed color over time.

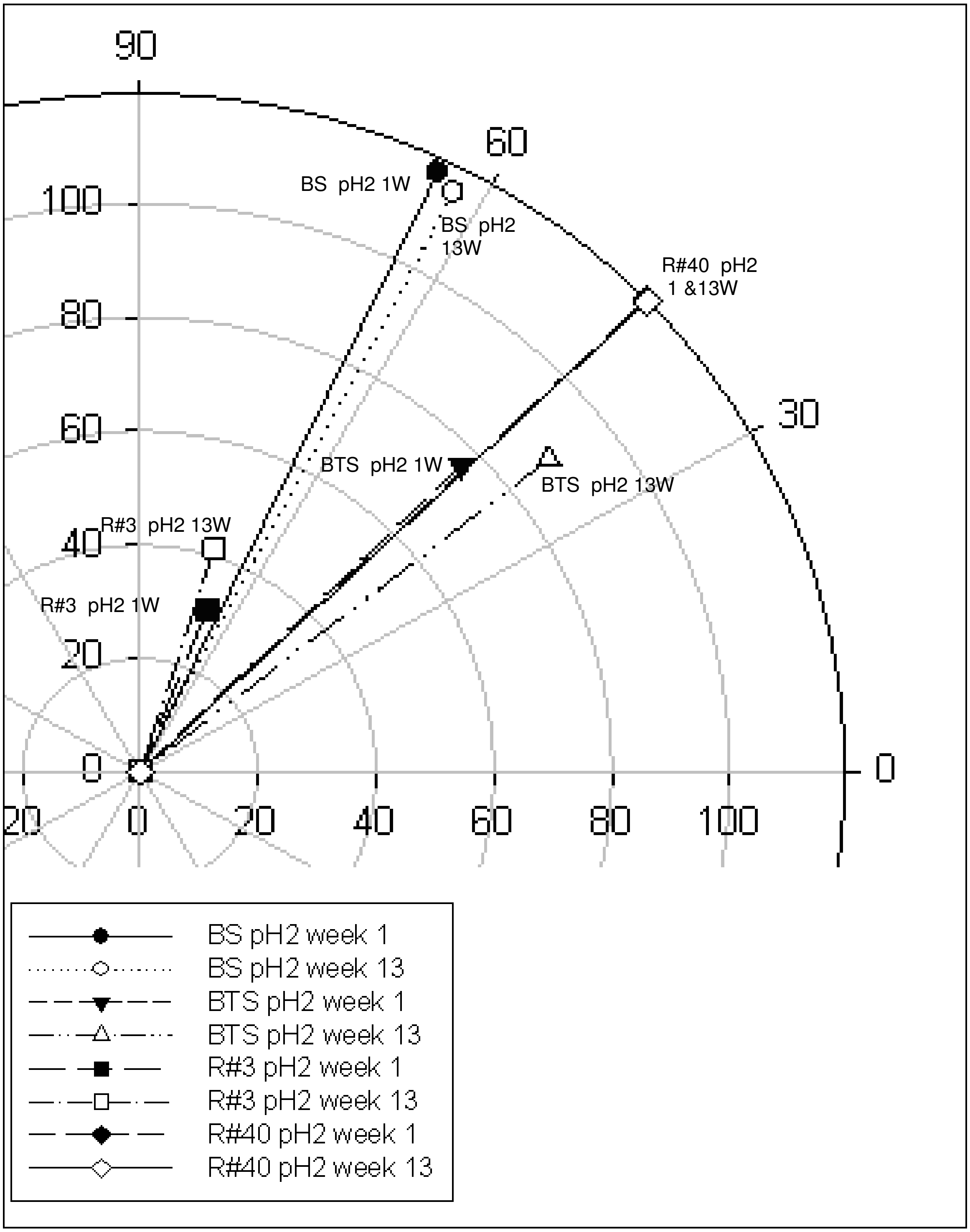


Fig. 19. Changes in color attributes of Tx430 Black (BS) and Black PI Tall (BTS) and standard Red No. 3(R#3) and Red No. 40(R#40) at pH 2, after 1 and 13 weeks. Plot represents the chroma and the hue.

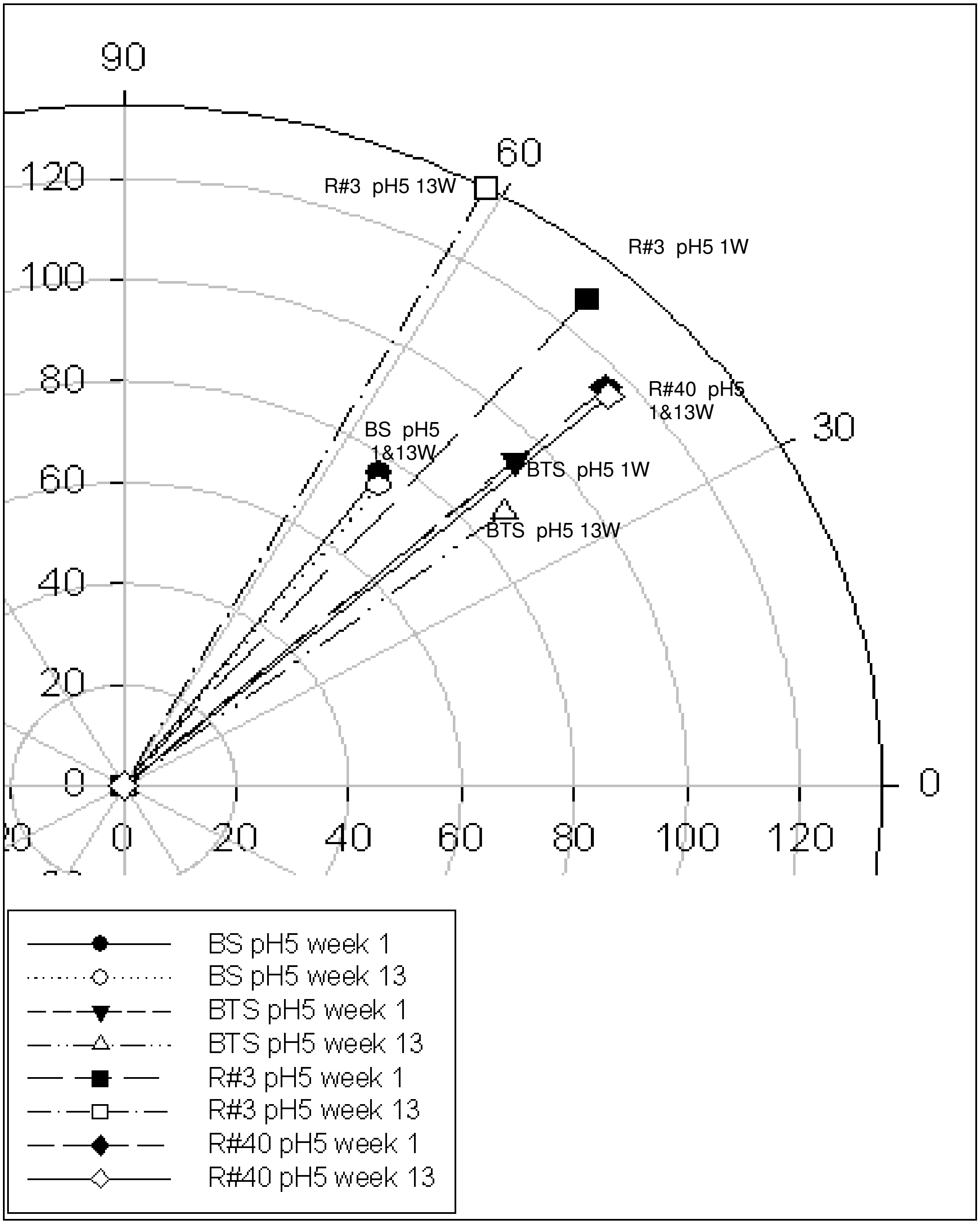


Fig. 20. Changes in color attributes of Tx430 Black (BS) and Black PI Tall (BTS) and standard Red No. 3(R#3) and Red No. 40(R#40) at pH 5, after 1 and 13 weeks. Plot represents the chroma and the hue.

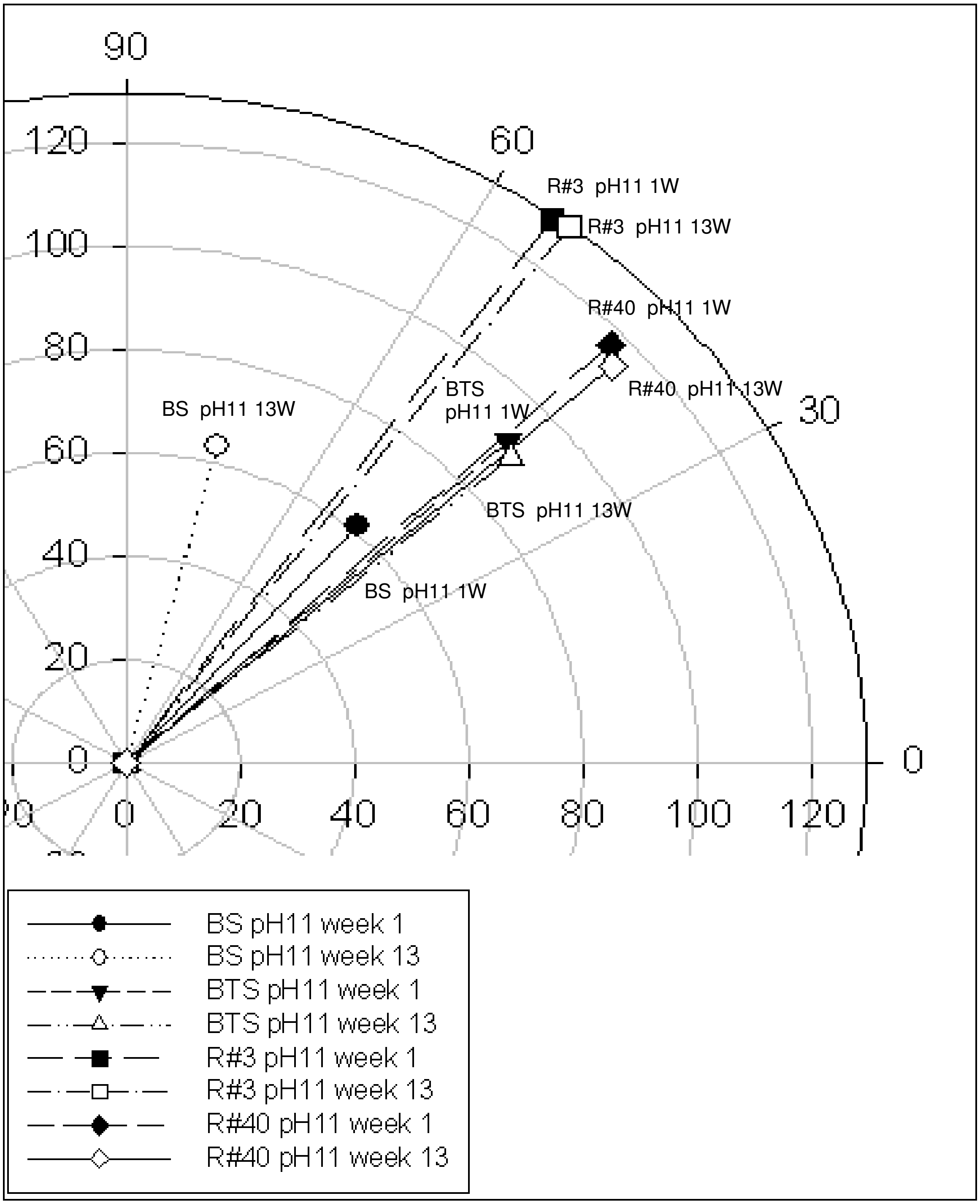


Fig. 21. Changes in color attributes of Tx430 Black (BS) and Black PI Tall (BTS) and standard Red No. 3(R#3) and Red No. 40(R#40) at pH 11, after 1 and 13 weeks. Plot represents the chroma and the hue.

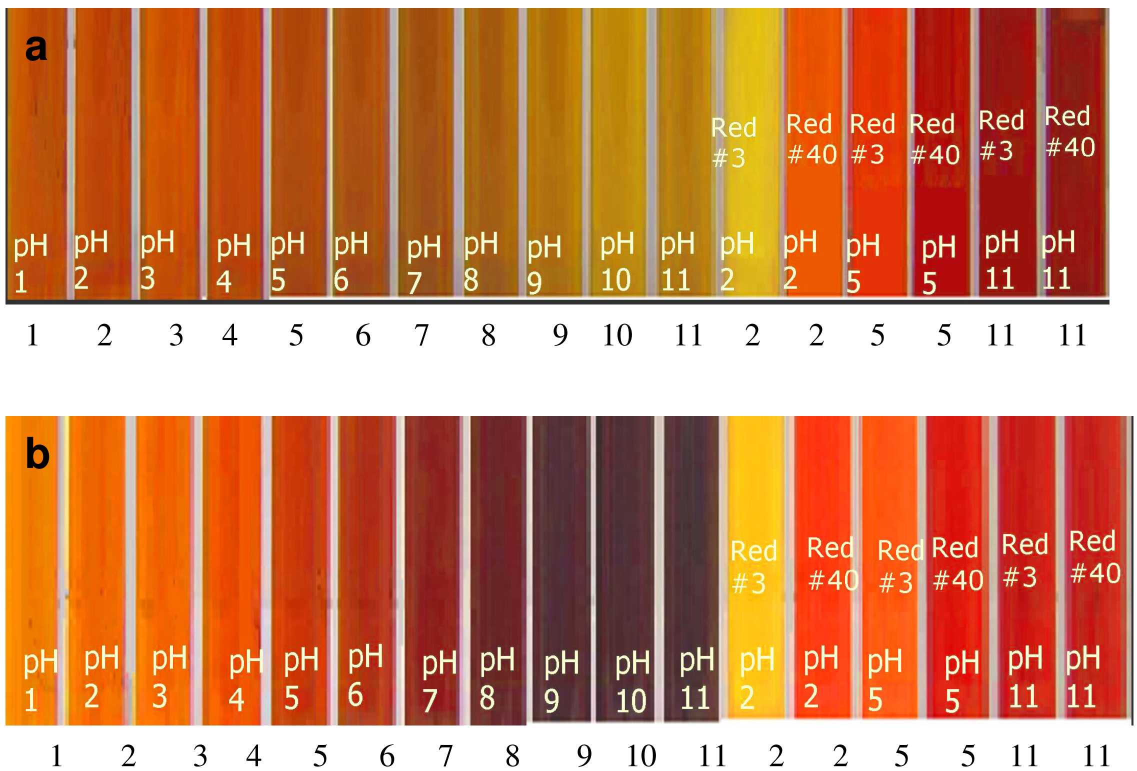


Fig. 22. Pictures of Tx430 Black sorghum bran extracted with 0.5% citric acid in 70% aqueous ethanol at different pH values. Concentration of 5 mg/mL at 25℃ after 1 week (a) and 13 weeks (b).

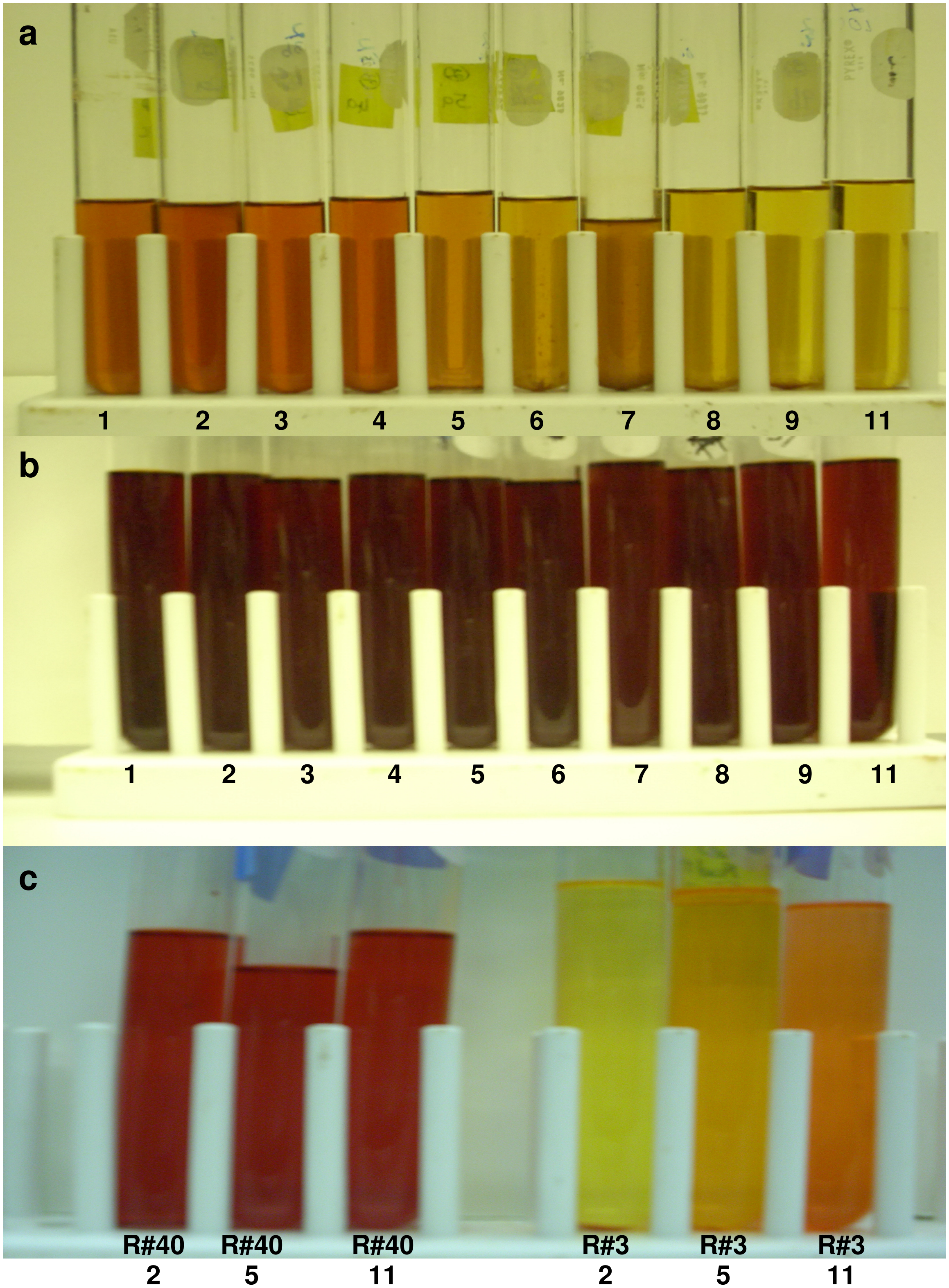


Fig. 23. Pictures of BS (a), BTS (b), and R#3 and R#40 (c) extracted with 0.5% citric acid in 70% aqueous ethanol at different pH values after 12 months. Concentration of 5 mg/mL at 25℃.

Temperature stability

Temperature affects the stability of anthocyanins and the rate of their degradation in natural and model systems. Structure of anthocyanins, pH, oxygen, and interactions with other components affect the thermal stability of anthocyanins. That is the reason that some studies showed that the factors that affect pH stability are the same that affect the temperature stability of the anthocyanins; the factors that increase pH stability of anthocyanin also increase their thermal stability (Hendry and Houghton, 1992). Removal of oxygen protects anthocyanins from thermal degradation (Rein, 2005).

An increase in temperature decreased color intensity because of anthocyanins degradation (Torres, 2002). In solutions of anthocyanins in which copigments were added, an increase in temperature provokes a strong reduction of color intensity (Mazza and Miniati, 1993). The mechanism of anthocyanin degradation is temperature-dependent; however the exact mechanism is not very clear. Three possible mechanisms were proposed. The first mechanism suggests that the flavylium cation is transformed to the quinonoidal base, then to intermediates, and finally to coumarin derivatives; the second mechanism suggests that the flavylium cation is transformed to a colorless carbinol base, then to the chalcone and finally to brown degradation products. The third mechanism is similar to the second one where the degradation products of chalcone are first inserted (Markakis, 1982). These three mechanisms suggest that thermal degradation of anthocyanins depends on the type of anthocyanin involved and the degradation temperature (Fennema, 1996).

The sorghum bran extracts at constant pH and concentration were exposed to different temperatures for a period of time. Lightness, chroma, and hue were monitored and the values were plotted and compared to Red #3 and Red #40.

Changes in visual color attributes of Tx430 Black sorghum bran extracts at different temperatures

BS at -8, 4, and 25°C were stable and followed the same trend (Fig. 24). They became brighter with time. Among the three samples, sorghum BS at 25°C was less bright but after two weeks, there were no statistical differences among them. Sorghum bran extract at 50°C had the lowest L-values of all sorghum samples and was the sample with more variation over time likely caused by evaporation of the solvent when exposed to high temperature. Red #3 was brightest at all temperatures. After 2 weeks, Red No. 3 at -8, 4, and 25°C were stable reaching L-values around 80 with no significant differences among the samples. Red #3 at 50°C increased lightness over time until it reached a stable state. This was a different trend than the rest of the temperatures. Red #40 showed the lowest L-values. These samples at -8, 4, and 25°C had the same behavior but Red No. 40 at 25°C had higher L-values than the rest. Red No. 40 at 50°C presented a very different trend; this sample was stable since the beginning of the experiment with L-values around 47.

The chroma values indicated that the BS had a vivid color (Fig. 25). BS at 50°C followed a different trend over time; C-values were decreasing and by the end of the experiment the C-values were below 100. At temperature of -8, 4 and

25°C the BS showed good stability after two weeks. The chroma values were high; between 110 and 116 with no significant differences between samples. Red No. 40 at all temperatures showed the same trend as the BS. C-values of Red No. 40 (114 and 122) were in the same range as the C-values of the BS. These data showed that the BS at -8, 25, and 50°C can compete with Red #40 in chroma values and stability. Red #3 was the most unstable at all temperatures. The dullest sample was at -8°C followed by the sample at 4°C. Red #3 at 25°C ended with C-values similar to the C-values of BS at 50°C. After one week, C-values of Red #3 at 50°C had the most vivid color with values above 150. BS at all temperatures had constant hue values over time (Fig. 26). When sorghum samples were stored at -8, 4, and 25°C, the hue became constant after two weeks; while at 50°C the hue became constant after 7 weeks.

The hue values of 64 correspond to orange in the color wheel. Red #3 at 25°C had orange color with values similar to BS. Red No. 3 at -4 and 8°C had lower values than BS, the values were around 55 and they were not significantly different. Red #3 at 50°C was the most unstable changing from orange to yellow-orange. Red #40 showed the lowest hue values; the color of the samples was orange-red. Red #40 had the most stable colors at all temperatures, followed by BS, however they had different hue.

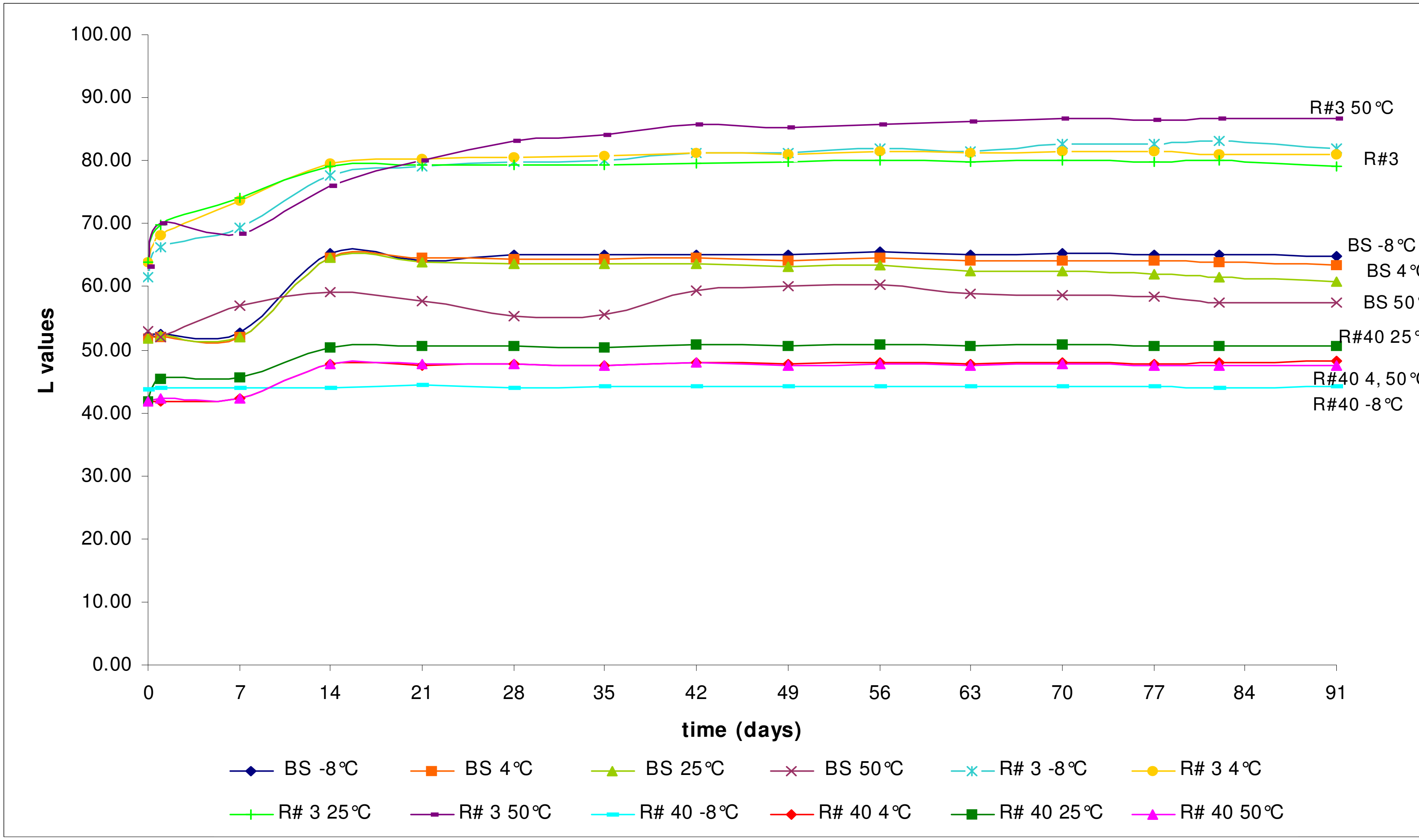


Fig. 24. Lightness values of Tx430 Black sorghum bran extracts (BS) and standard Red No.3 (R# 3) and Red No.40 (R#40) over time at different temperatures. Concentration of 5mg of extract/mL of citric acid in aqueous ethanol at pH 2 kept in the dark.

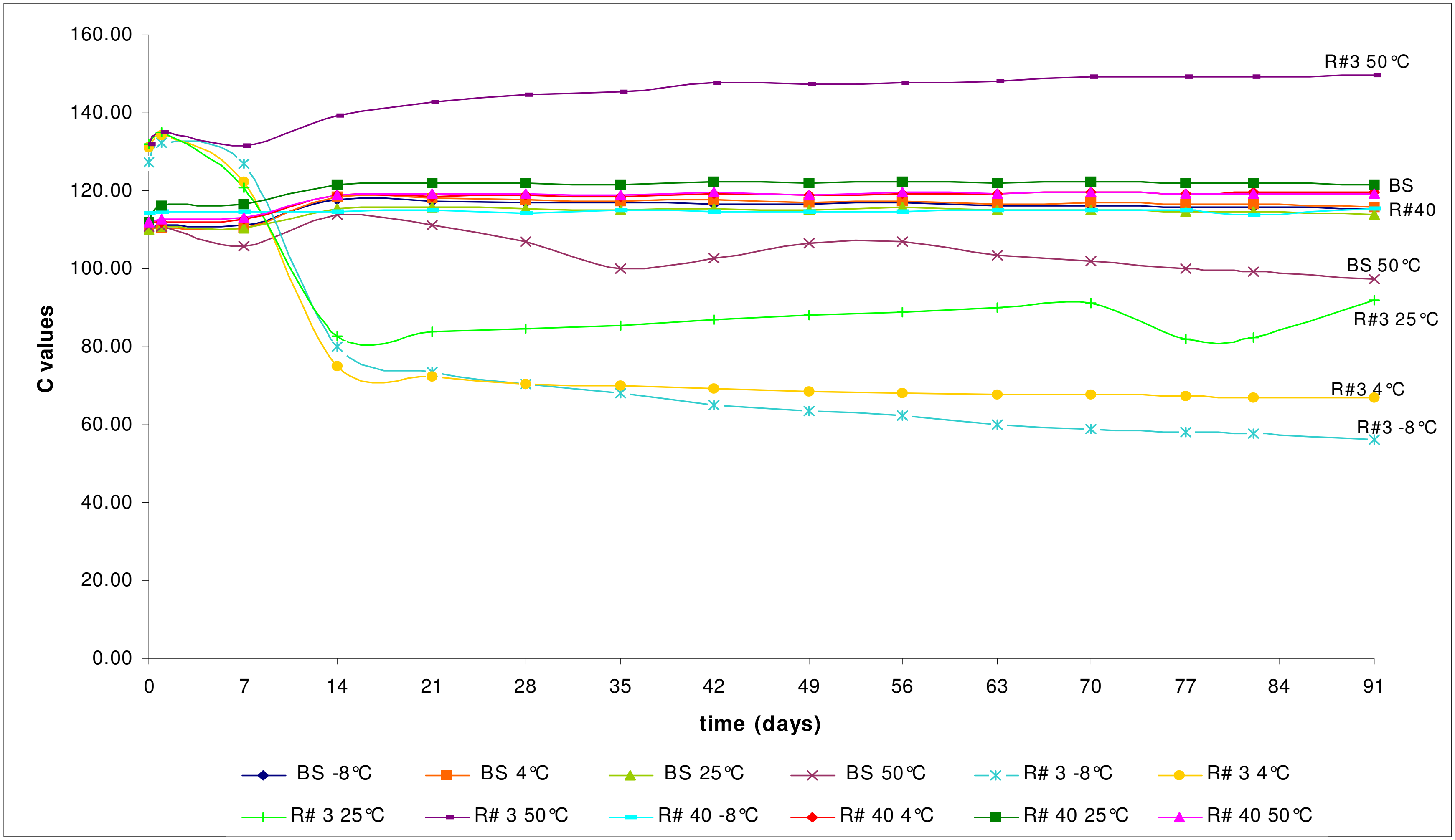


Fig. 25. Chroma values of Tx430 Black sorghum bran extracts (BS) and standard Red No.3 (R# 3) and Red No.40 (R#40) over time at different temperatures. Concentration of 5mg of extract/mL of citric acid in aqueous ethanol at pH 2 kept in the dark.

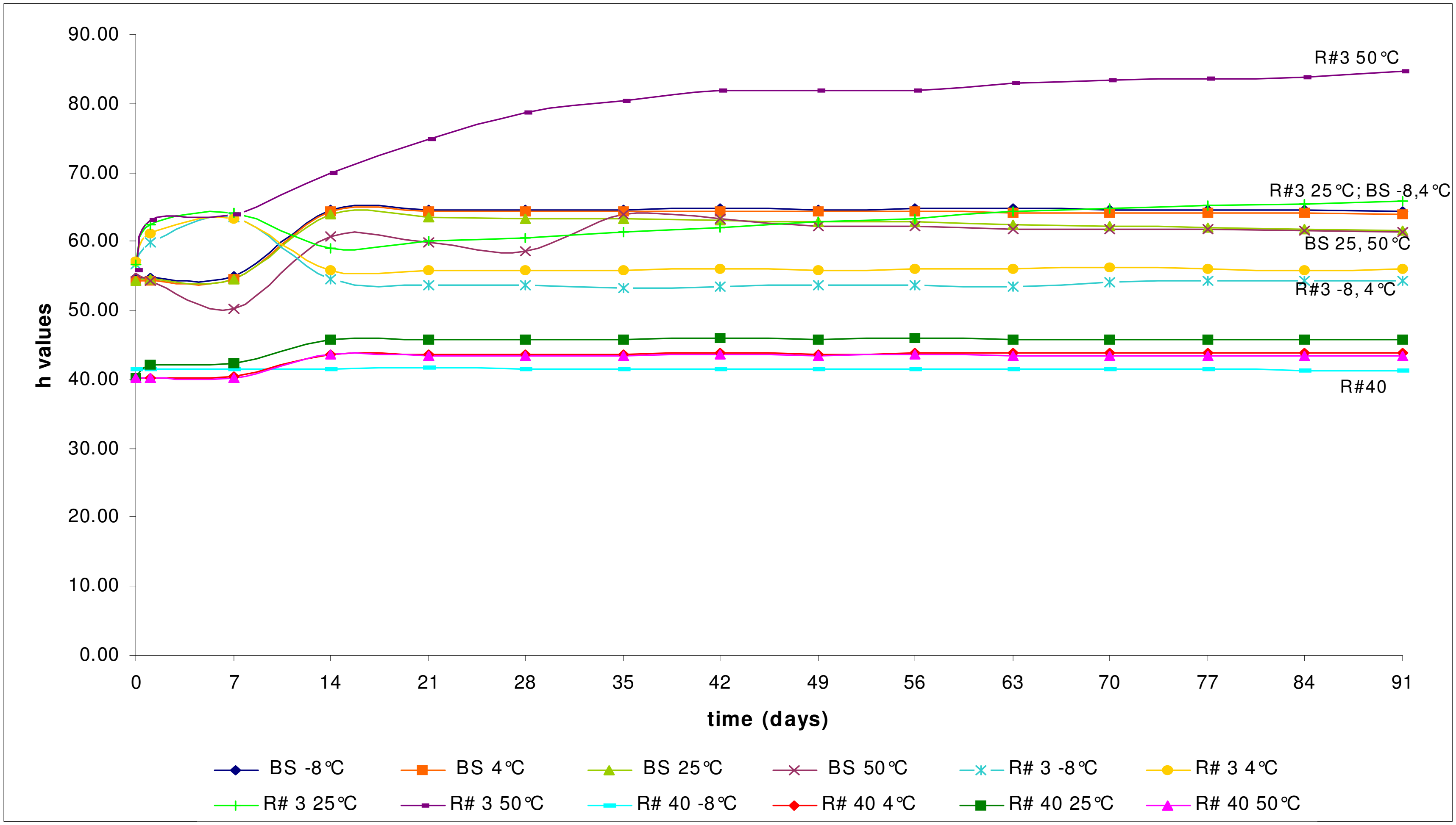


Fig. 26. Hue values of Tx430 Black sorghum bran extracts (BS) and standard Red No.3 (R# 3) and Red No.40 (R#40) over time at different temperatures. Concentration of 5mg of extract/mL of citric acid in aqueous ethanol at pH 2 kept in the dark.

Changes in visual color attributes of Black PI Tall sorghum bran extracts at different temperatures

BTS had the lowest L-values, meaning that these samples had darker color (Fig. 27). L-values of BTS at -8℃ were significant stable through time. After 1 week, sorghum samples at 4℃ were stable and whereas those at 25ºC were stable after 5 weeks. BTS at 50℃ had a big change through time; L-values decreased until they got L-values close to 30. Red #40 at all temperatures had values between 45 and 50. The highest L-values were shown by the temperature of 25℃. Red #40 at -8℃ was very stable and the rest of the samples were stable after 2 weeks. L-values of these samples were higher than those of BTS. The brightest samples were the standard Red #3 at all temperatures. All of them but the one at 50℃ were stable after 2 weeks.

The chroma values of the BTS at -8 and 4℃ were constant and stable over time (Fig. 28). Samples at 25 and 50ºC were stable after two and 6 weeks, respectively. The chroma values of the black sorghum were between Red #3 and Red #40. Red #40 was stable after two weeks showing vividness in the color. Red #3 samples were unstable.

The hue of BTS stored at -8, 4, and 25℃ was stable over time (Fig. 29). Thus, BTS were good replacements for Red #40 because they were not significantly different. BTS at 50℃ was different from the rest of the samples over time. This sample had the lowest h-values, with a change of color from orange to red color. Red #3 had the highest h-values but at 50℃ changed over time.

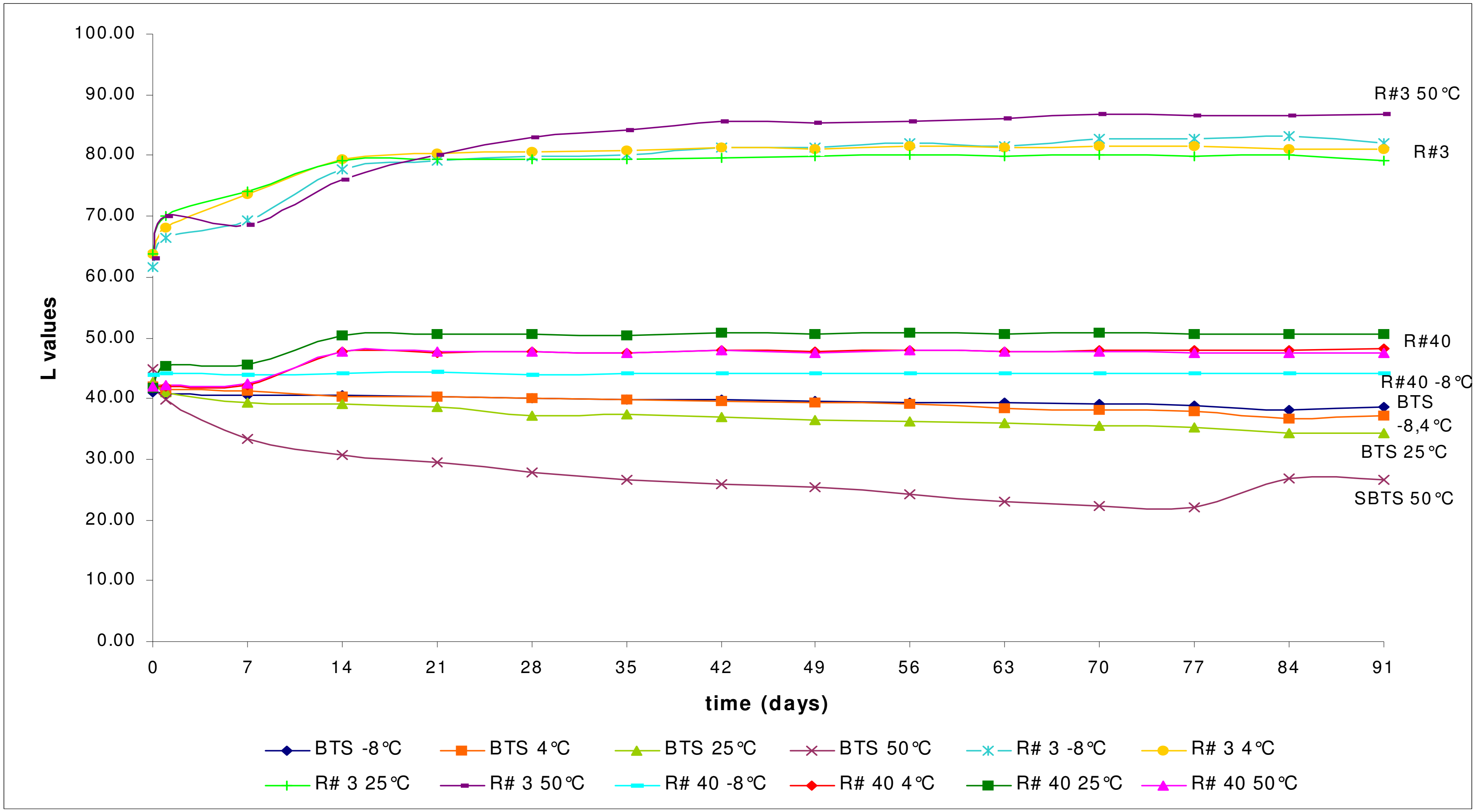


Fig. 27. Lightness values of Black PI Tall sorghum bran extracts (BTS) and standard Red No.3 (R# 3) and Red No.40 (R#40) over time at different temperatures. Concentration of 5mg of extract/mL of citric acid in aqueous ethanol at pH 2 kept in the dark.

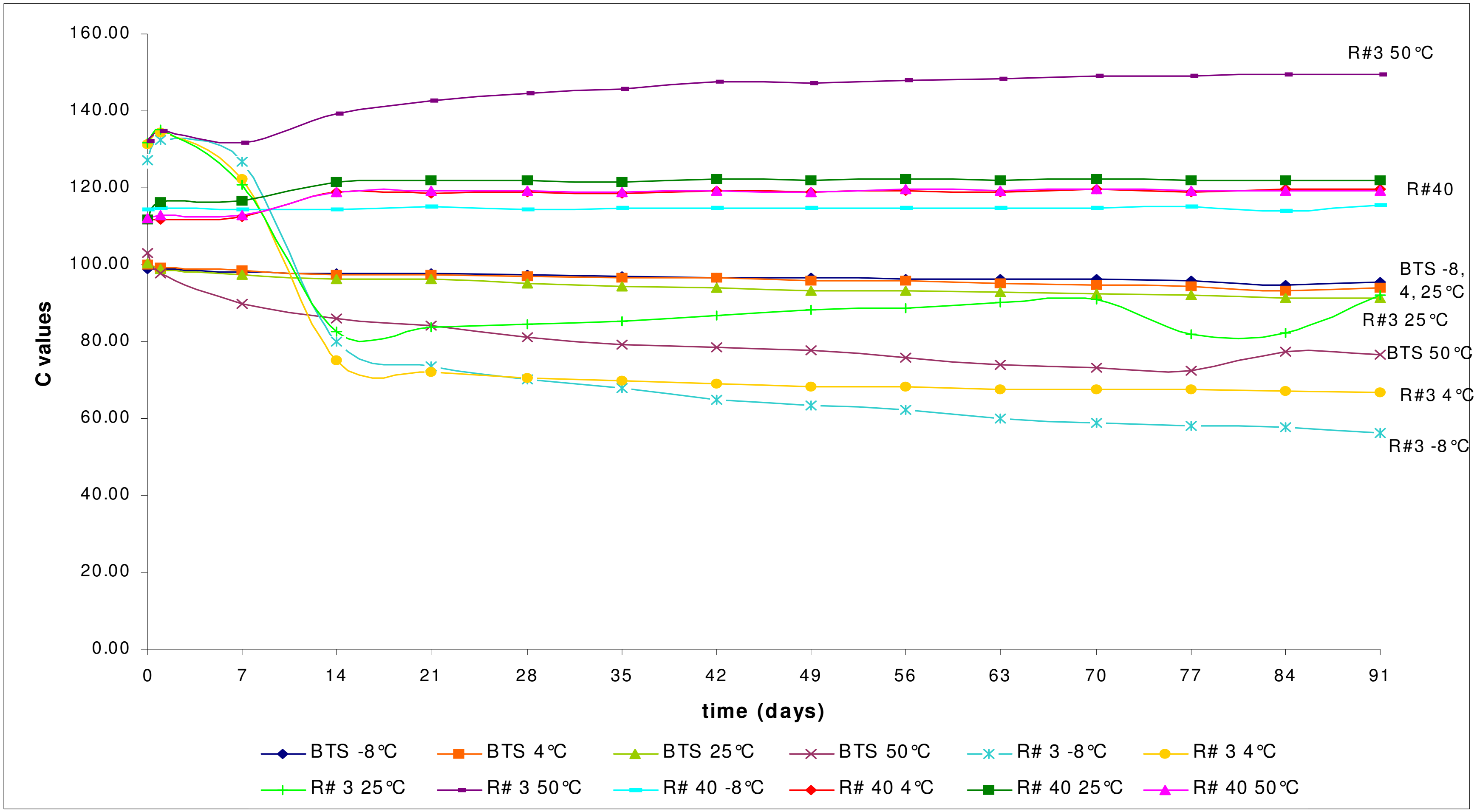


Fig. 28. Chroma values of Black PI Tall sorghum bran extracts (BTS) and standard Red No.3 (R# 3) and Red No.40 (R#40) over time at different temperatures. Concentration of 5mg of extract/mL of citric acid in aqueous ethanol at pH 2 kept in the dark.

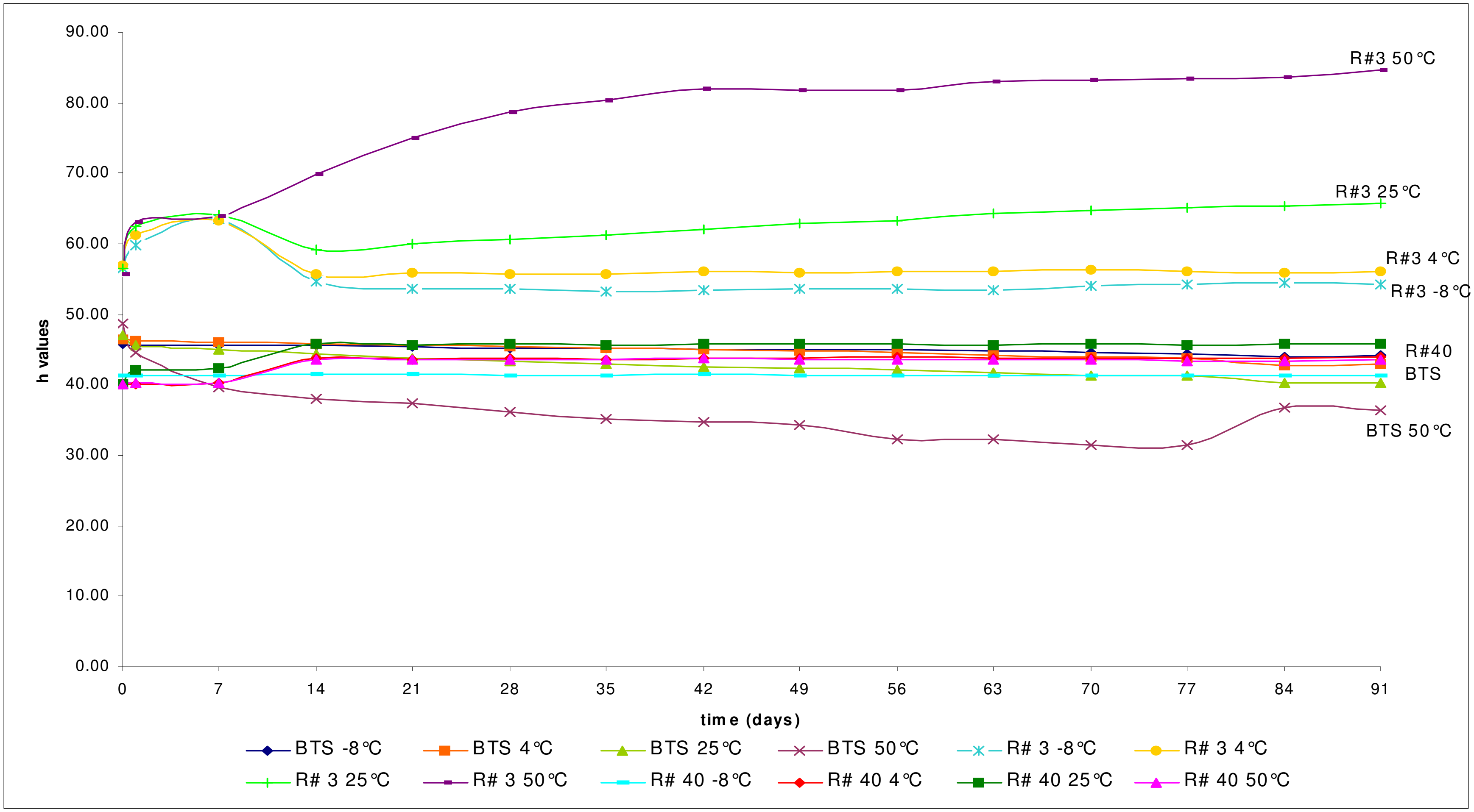


Fig. 29. Hue values of Black PI Tall sorghum bran extracts (BTS) and standard Red No.3 (R# 3) and Red No.40 (R#40) over time at different temperatures. Concentration of 5mg of extract/mL of citric acid in aqueous ethanol at pH 2 kept in the dark.

The chemical structure of the anthocyanins present in extracts of purple and red-flesh potato caused differences in temperature stability (Reyes and Cisneros-Zevallos, 2007). The chroma values of some berries decreased significantly with a loss of color intensity when temperature increased and the hue values increased after prolonged exposure to heat (Sadilova et al., 2006). The structure of the 3-deoxyanthocyanins and other anthocyanins could be caused by changes in the structure.

Degradation rate of anthocyanins increases during processing and storage as temperature rises. With time, thermal degradation of anthocyanins leads to brown products (Rein, 2005), especially in the presence of oxygen (Bridle and Timberlake, 1996). In a study using amaranthus anthocyanins in jelly, increasing storage temperature and extending storage time gave jelly samples low color retention as the degradation rate increased. When the amaranthus anthocyanins were used in beverages stored at low temperatures (4°C), the L, C, and h values were stable over twenty weeks, but at 25°C, deterioration was significant after 12 weeks (Cai and Corke, 1999).

According to Bridle and Timberlake (1996), anthocyanins become paler during heating because the equilibrium between the four structures of anthocyanin shifts to the colorless forms. Under perfect conditions, the original color should be recovered on cooling if sufficient time is allowed for the reconversion.

Temperature also affects copigmentation. Increments in time and heating temperature change the anthocyanins and copigment contents as well as the copigmentation formation, which causes a change in color (Bakowska et al., 2003). In a study performed by Gradinaru et al. (2003) with hibiscus colorant, the extracts of the hibiscus were treated with copigments. Copigments help to improve the stability of the hibiscus extracts. But in this study copigments did not showed an effect on stability because at high temperature the copigment is inhibited.

Color changes at two different temperatures are plotted in Figs. 30, 31, 32, and 33. At 25°C, BTS have similar hue and chroma values as Red #40. BS had similar values as those of Red #3 but over time the hue values of BS decreased becoming similar to Red #40. When extracts where under 50°C, BTS were comparable to Red #40. The color of BS was between Red #3 and Red #40. BS and Red #3 changed in color over time.

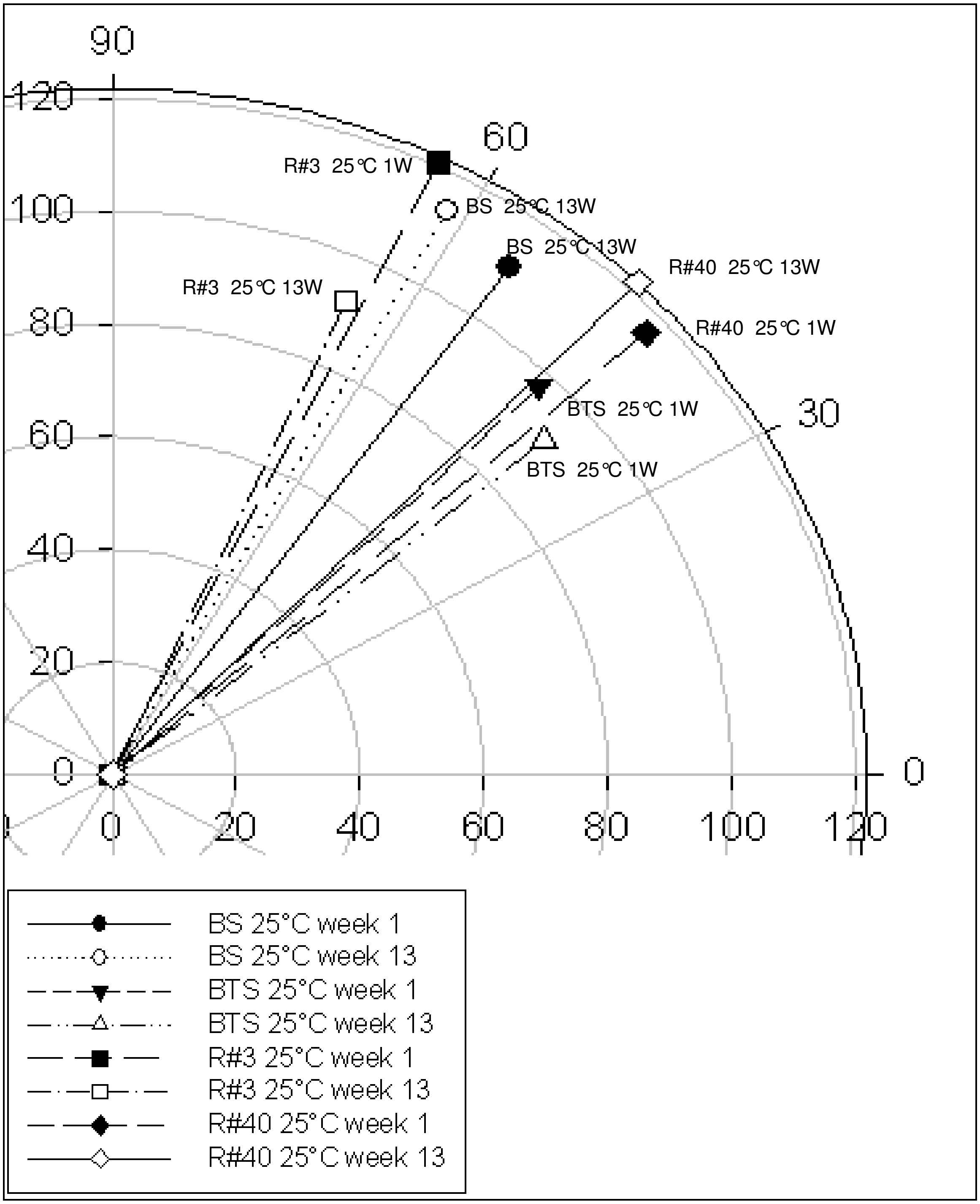


Fig. 30. Changes in color attributes of Tx430 Black (BS) and Black PI Tall (BTS) and standard Red No. 3(R#3) and Red No. 40(R#40) at 25℃, after 1 and 13 weeks. Plot represents the chroma and the hue.

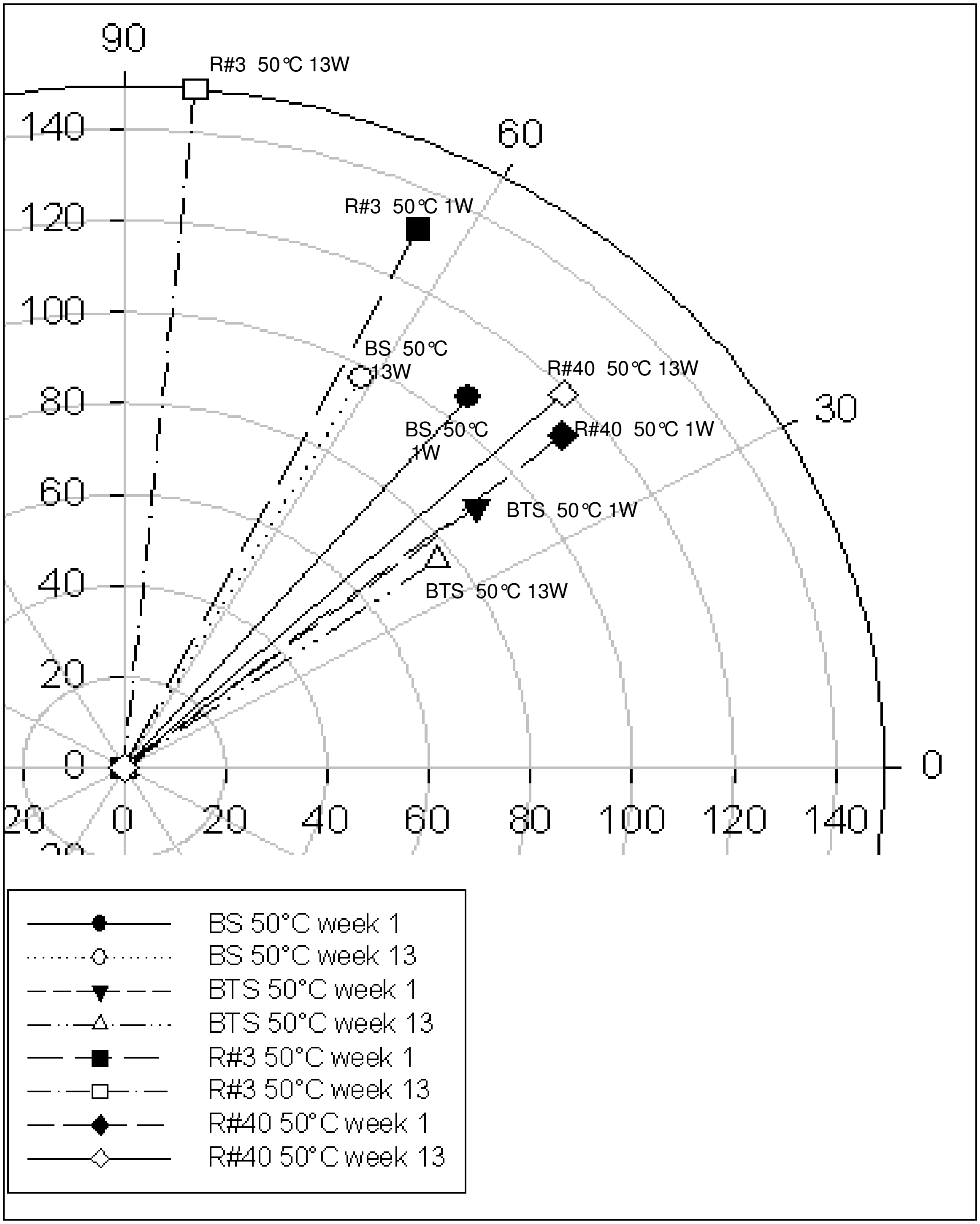
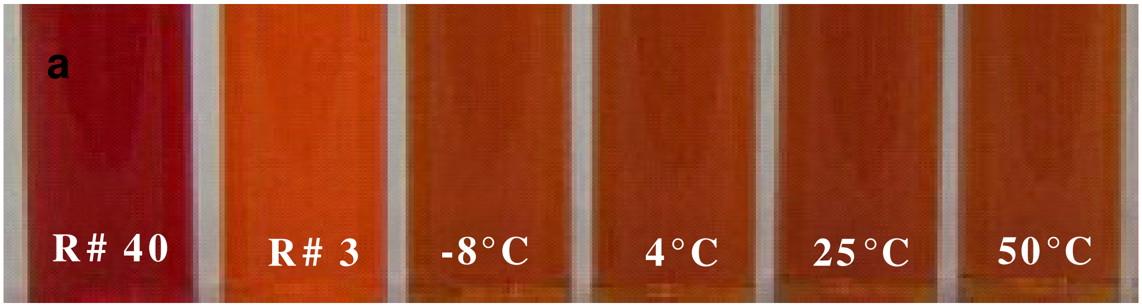


Fig. 31. Changes in color attributes of Tx430 Black (BS) and Black PI Tall (BTS) and standard Red No. 3(R#3) and Red No. 40(R#40) at 50℃, after 1 and 13 weeks. Plot represents the chroma and the hue.



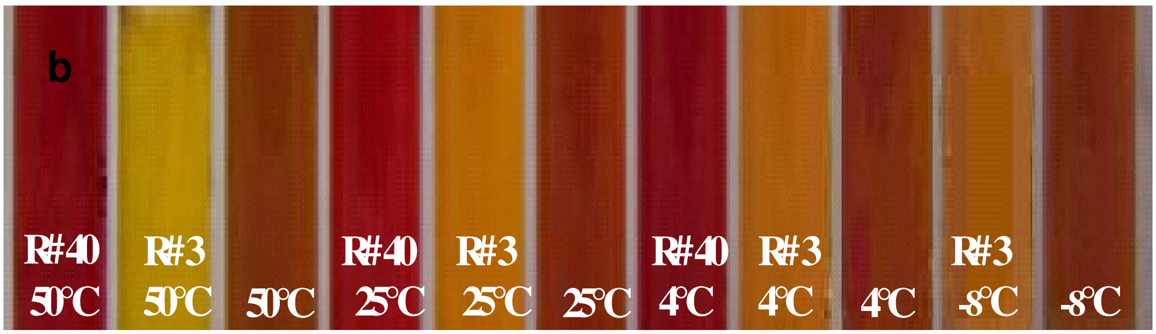


Fig. 32. Pictures of Tx430 Black sorghum bran extracted with 0.5% citric acid in 70% aqueous ethanol at different temperatures. Concentration of 5 mg/mL at pH 2 after 1 week (a) and 13 weeks (b).

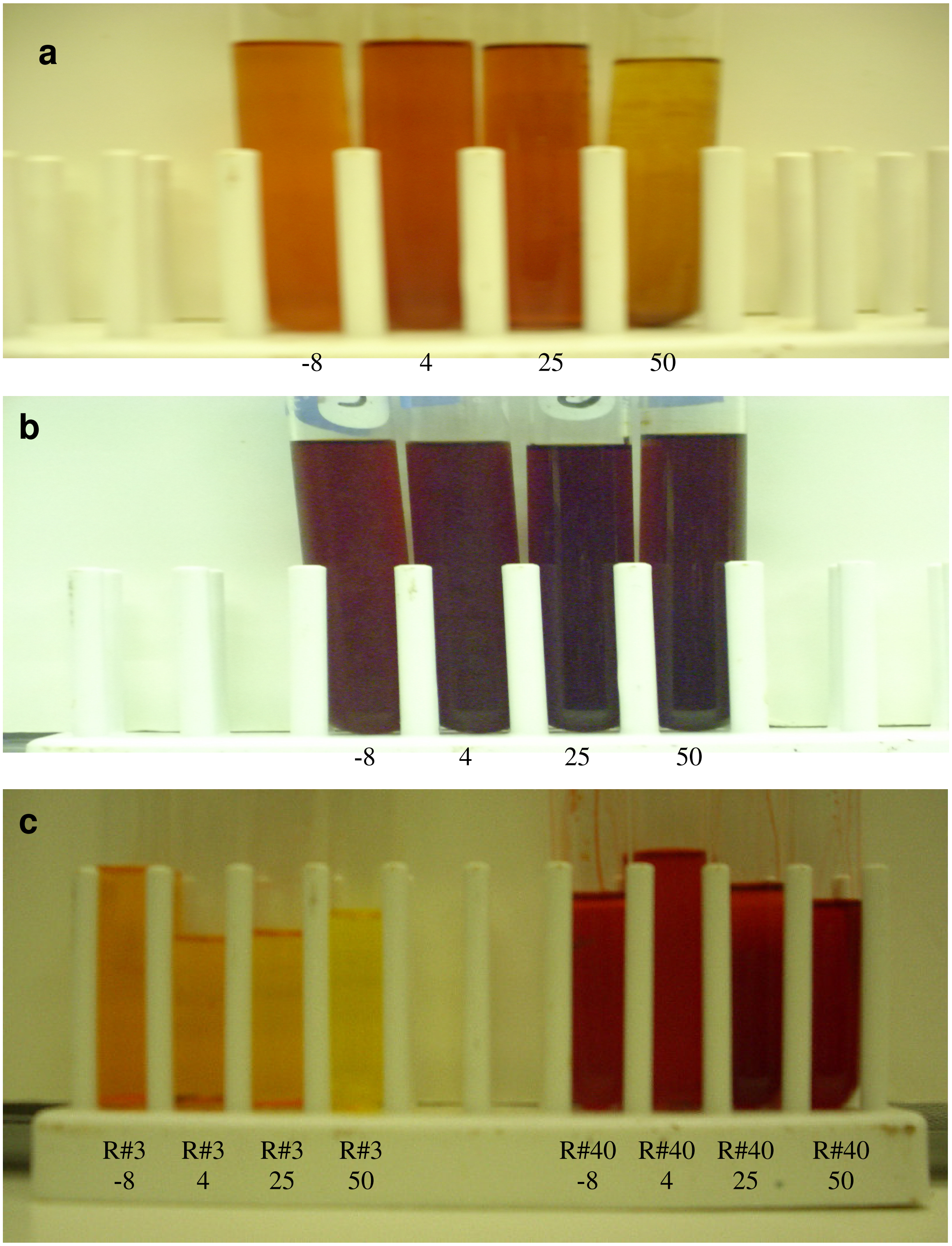


Fig. 33. Pictures of BS (a), BTS (b), and R#3 and R#40 (c) extracted with 0.5% citric acid in 70% aqueous ethanol at different pH values after 12 months. Concentration of 5 mg/mL at pH 2.

Water activity stability

Anthocyanin stability increases when water activity decreases. Wrolstad (2000) found that anthocyanin colorants were better in intermediate and low-moisture food systems.

When sorghum bran extracts were dissolved in water, precipitation occurred and the solutions became cloudy. This was probably due tu polymeric pigments present in the ethanolic extracts. The water activity of the aqueous anthocyanin extracts was modified with different levels of sucrose and glycerol.

Sucrose was selected to change water activity of the aqueous sorghum bran extracts because this component at high concentration stabilizes anthocyanins by lowering water activity. Reduction of water activity is associated with a reduction in degradation rate of anthocyanins (Hendry and Houghton, 1992). On the other hand, when the concentration is too low to affect water activity, the degradation of the sugars can accelerate the degradation of anthocyanins (Fennema, 1996). The change to colorless forms become less favorable when water is limited (Hendry and Houghton, 1992).

Some sugars have a strong degradative effect on anthocyanins. At low concentrations, fructose, arabinose, lactose and sorbose have a greater degradative effect on anthocyanins than glucose, sucrose, and maltose (Fennema, 1996). Oxygen accelerated the degradative effect (Markakis, 1982).

Changes in visual color attributes of Tx430 Black sorghum bran extracts at different water activities

BS had the highest L-values (Fig. 34). The samples in 0.20 water activities (a_w) had the highest values, followed by the water activities of 0.85 and 0.95. Samples in a_w of 1.0, 0.95, and 0.85 were constant over time. Sorghum BS in a_w 0.20 become brighter over time; 76.5 was the initial L-value and after 7 weeks reached 90. Sorghum samples in a_w 0.95 and 0.85 had similar values (between 73 at the beginning and 82 at the end of the experiment). BS at 1.0 initially had similar behavior to extracts at 0.95 and 0.85 extracts but with time, precipitation appeared and the samples were no longer homogenous. Red #40 at all water activities measured was the darkest. After 1 day, the samples had constant L-values over time and no significant differences were found between samples; the values were between 50 and 55. The lightness of Red #3 was between the BS and Red #40. These samples were constant over the 13 weeks of the experiment.

The chroma values of BS were the lowest (Fig. 35). All sorghum samples become less vivid with time. The most noticeable change was at a_w of 1.0 where C-values decreased over time by 20 units; the C-values of the sample in a_w of 0.20 decreased by 30 units. BS in a_w 0.95 and 0.85 followed the same trend. They showed high C-values and by the seventh week there were no significant differences between these two samples. Red #40 at all water activities had the most vivid colors. After one day, the C-value remained constant. The chroma values of Red #3 at all water activities were between those of Red #40

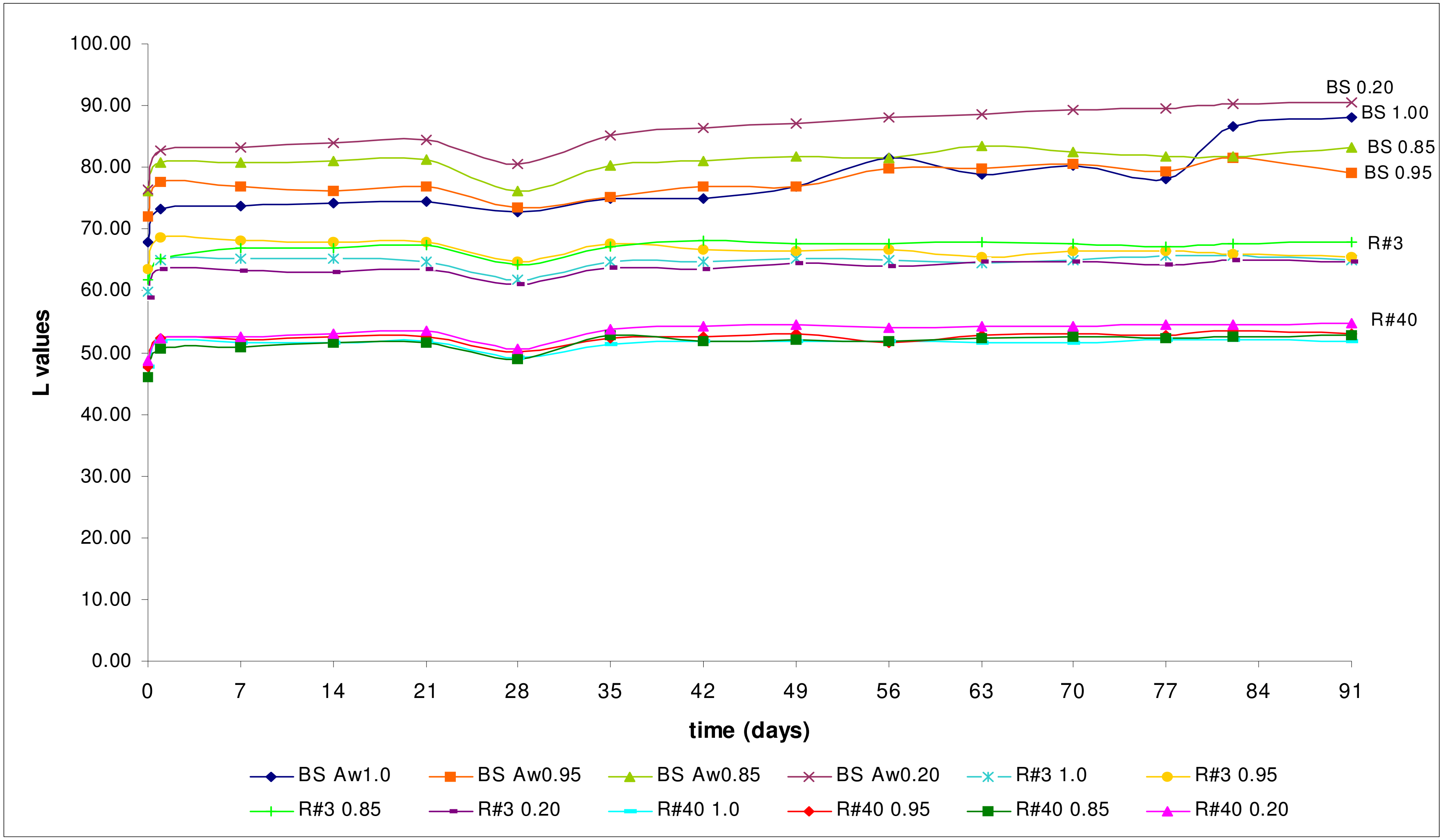


Fig. 34. Lightness values of Tx430 Black sorghum bran extracts (BS) and standard Red No.3 (R# 3) and Red No.40 (R#40) over time at different a_w. Concentration of 5mg of extract/mL of citric acid in aqueous ethanol at pH 2 and 25℃ kept in the dark.

and BS. The values did not change over time. The sample with the highest values was the sample in a_w of 1.0; those in a_w of 0.85 and 0.95 activities had the lowest values.

BS showed the highest hue values (Fig. 36). These samples were in the yellow-orange region. The extracts were stable during all the experiments. The hue values of the sorghum bran extract in a_w 0.95 and 0.85 were very similar. The hue values of Red #40 at all water activities did not change over time. The color of the samples were orange (hue values around 47-48); there were no significant differences among these four samples. The hue values of Red #3 at all water activities were constant over time but they were significantly different among them except for those with water activities of 0.85 and 0.95. Red #3 in a_w of 0.20 showed the lowest value (around 30).

and therefore the color was orange-red. BS was not comparable with Red #3 and Red #40 when the parameters L, C, and h were compared in terms of water activity.

Changes in visual color attributes of Black PI Tall sorghum bran extracts at different water activities

When the water activity of BTS increased, L-values decreased (Fig. 37). The L-values of BTS with 0.20 water activity did not change with time. BTS in a solution of 1.0 water activity were darkest; the L-values were constant over time. BTS in a_w of 0.85 have variation over time; the L-values were around 51. Red #40 at all water activities had good stability. L-values were not significantly different among the samples. Thus, BTS in a_w of 0.85 are comparable with Red

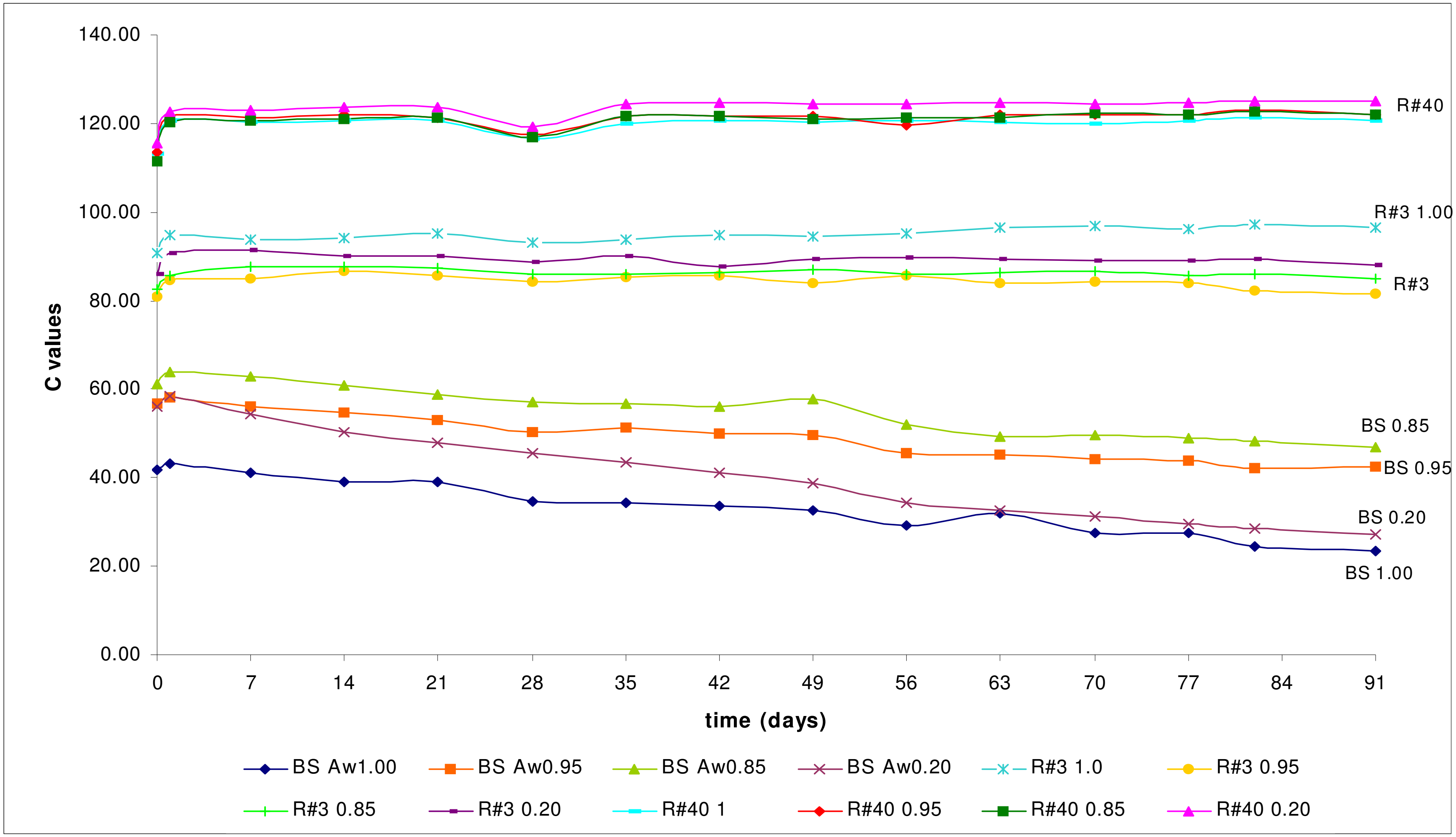


Fig. 35. Chroma values of Tx430 Black sorghum bran extracts (BS) and standard Red No.3 (R# 3) and Red No.40 (R#40) over time at different a_w. Concentration of 5mg of extract/mL of citric acid in aqueous ethanol at pH 2 and 25℃ kept in the dark.

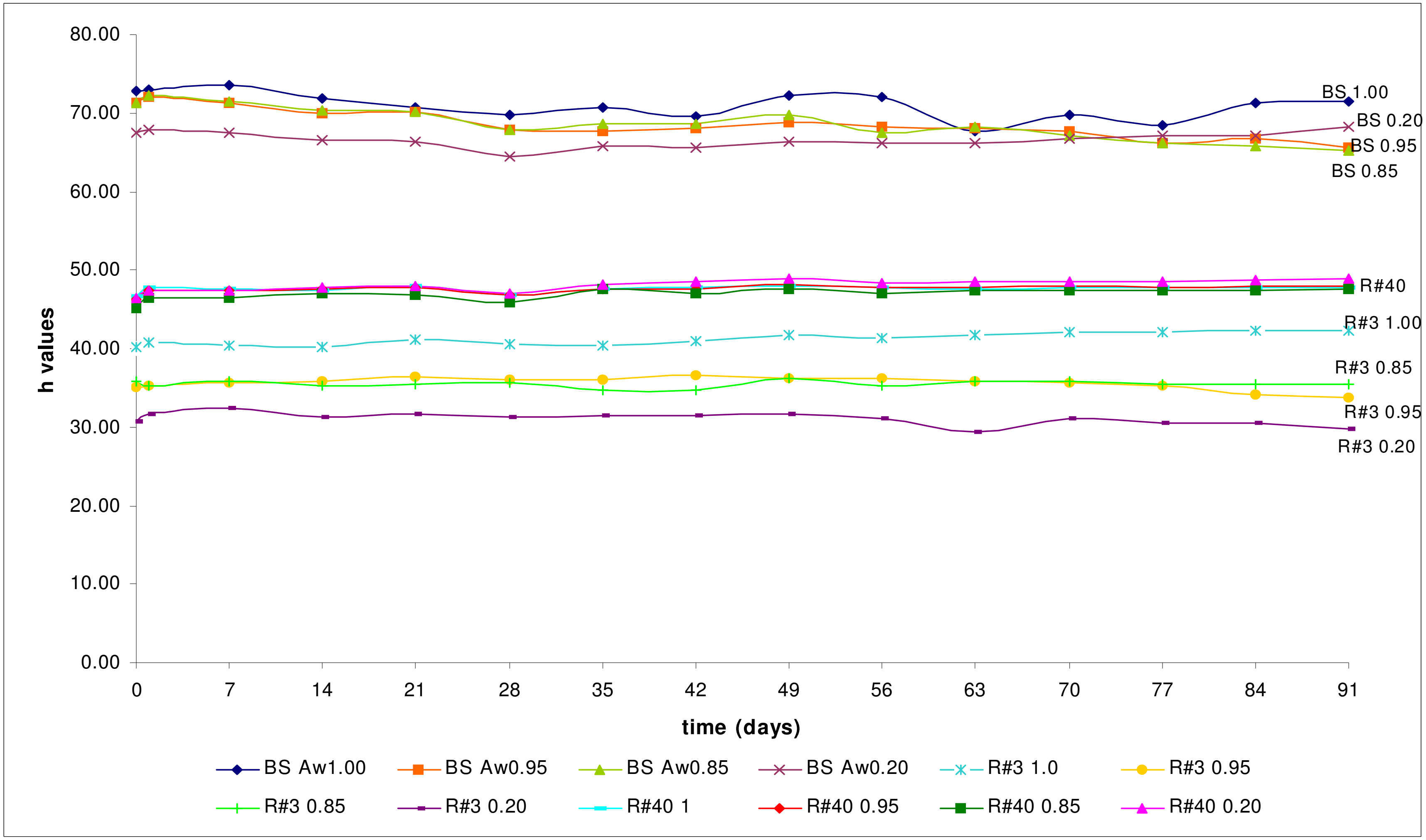


Fig. 36. Hue values of Tx430 Black sorghum bran extracts (BS) and standard Red No.3 (R# 3) and Red No.40 (R#40) over time at different a_w. Concentration of 5mg of extract/mL of citric acid in aqueous ethanol at pH 2 and 25℃ kept in the dark.

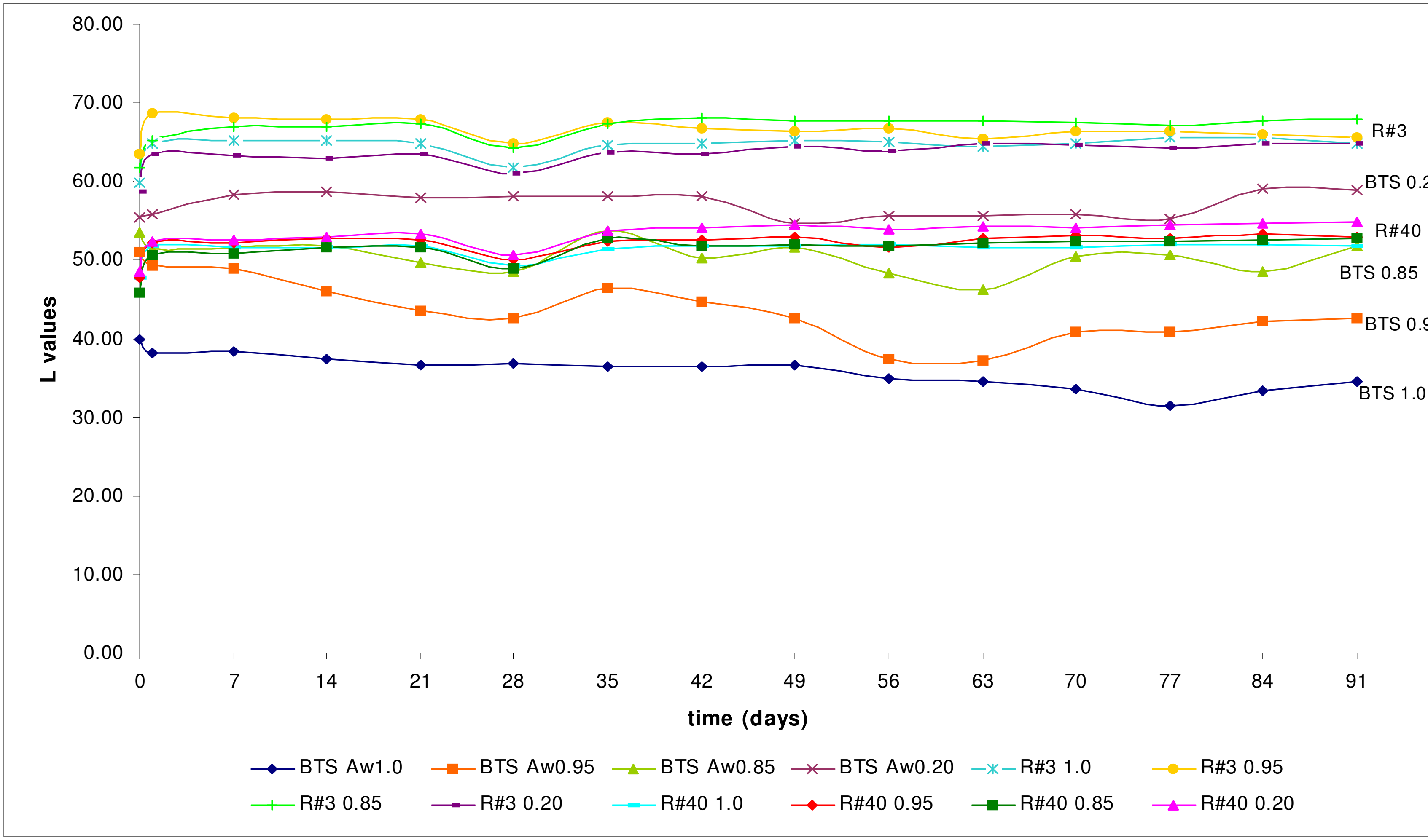


Fig. 37. Lightness values of Black PI Tall sorghum bran extracts (BTS) and standard Red No.3 (R# 3) and Red No.40 (R#40) over time at different a_w. Concentration of 5mg of extract/mL of citric acid in aqueous ethanol at pH 2 and 25℃ kept in the dark.

#40; after 7 weeks BTS in a_w of 0.20 were comparable with the standard. Red #3 at all water activities had the highest values. After 1 day, L-values were between 64 and 68 showing good stability over time.

When water activity of BTS increased C-values decreased (Fig. 38). Sorghum bran extract in a_w of 0.20 was constant over time with the highest C-values among the rest of BTS. The C-values of the samples in a_w of 1.00 remain constant over time with the dullest color. Red #3 in a_w of 0.95 and 0.85 was constant over time with C-values of around 85. After 2 weeks, BTS at water activity of 0.95 were comparable with Red #3 at those two water activities. Red #3 in a_w 1.00 and 0.20 did not change with time. The most vivid colors represented with the highest C-values correspond to Red #40. After one day, the standard at all water activities studies were stable with values above 120.

As the water activity decreased, the extracts become more orange-yellow (Fig. 39). BTS in a_w of 0.20 and 0.85 did not vary with time and the color was orange. After 3 weeks, BTS in a_w of 0.95 vary over time with values around 55. On the other hand, BTS in a_w of 1.00 were not stable over time and the hue decreased to the orange-red region. Red #3 at 0.20 water activity had the lowest values just above 30, showing a red-orange color; the hue values were constant. The hue at all water activities of Red #40 was constant over time with values between BTS and Red #3.

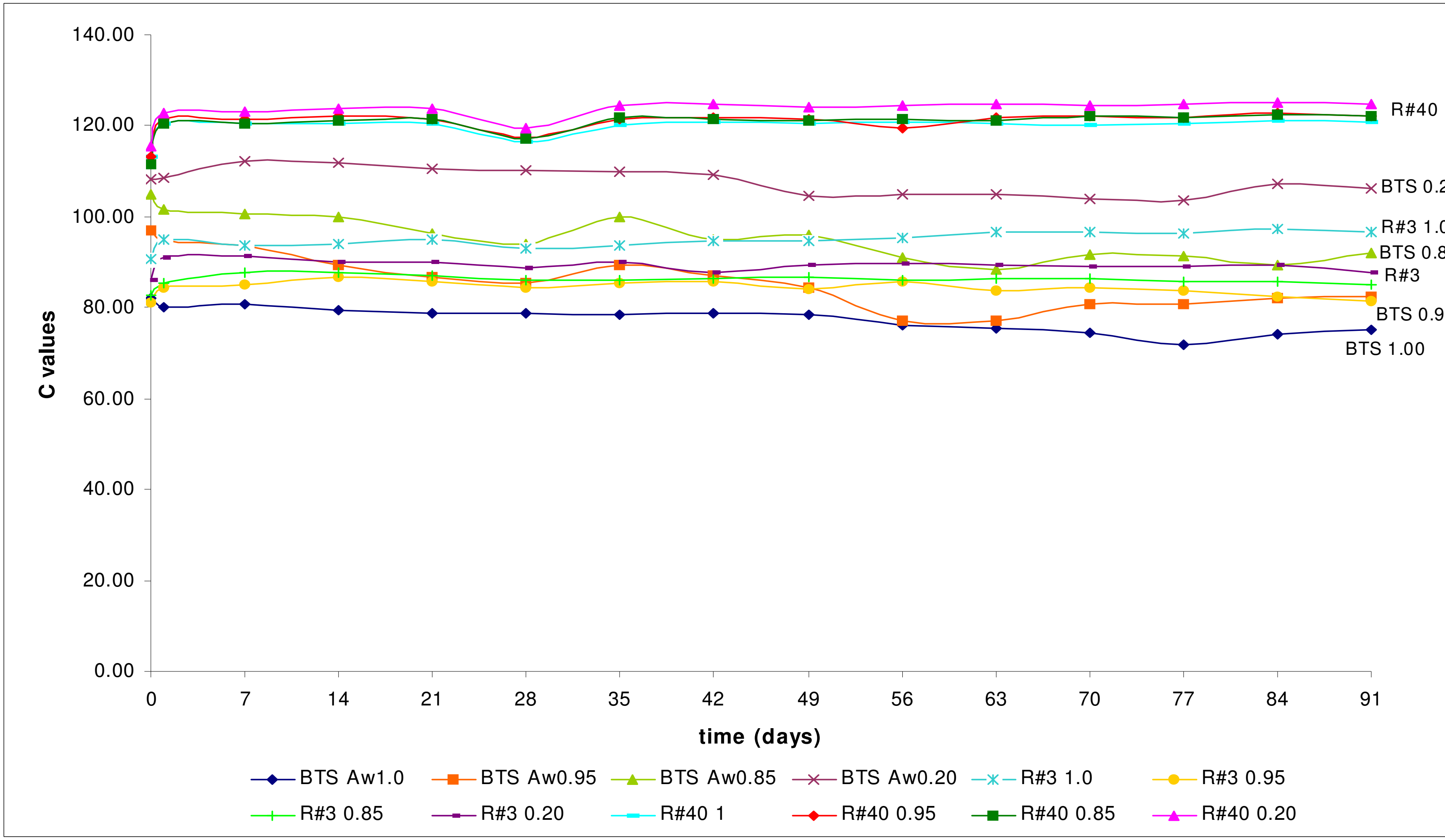


Fig. 38. Chroma values of Black PI Tall sorghum bran extracts (BTS) and standard Red No.3 (R# 3) and Red No.40 (R#40) over time at different a_w. Concentration of 5mg of extract/mL of citric acid in aqueous ethanol at pH 2 and 25℃ kept in the dark.

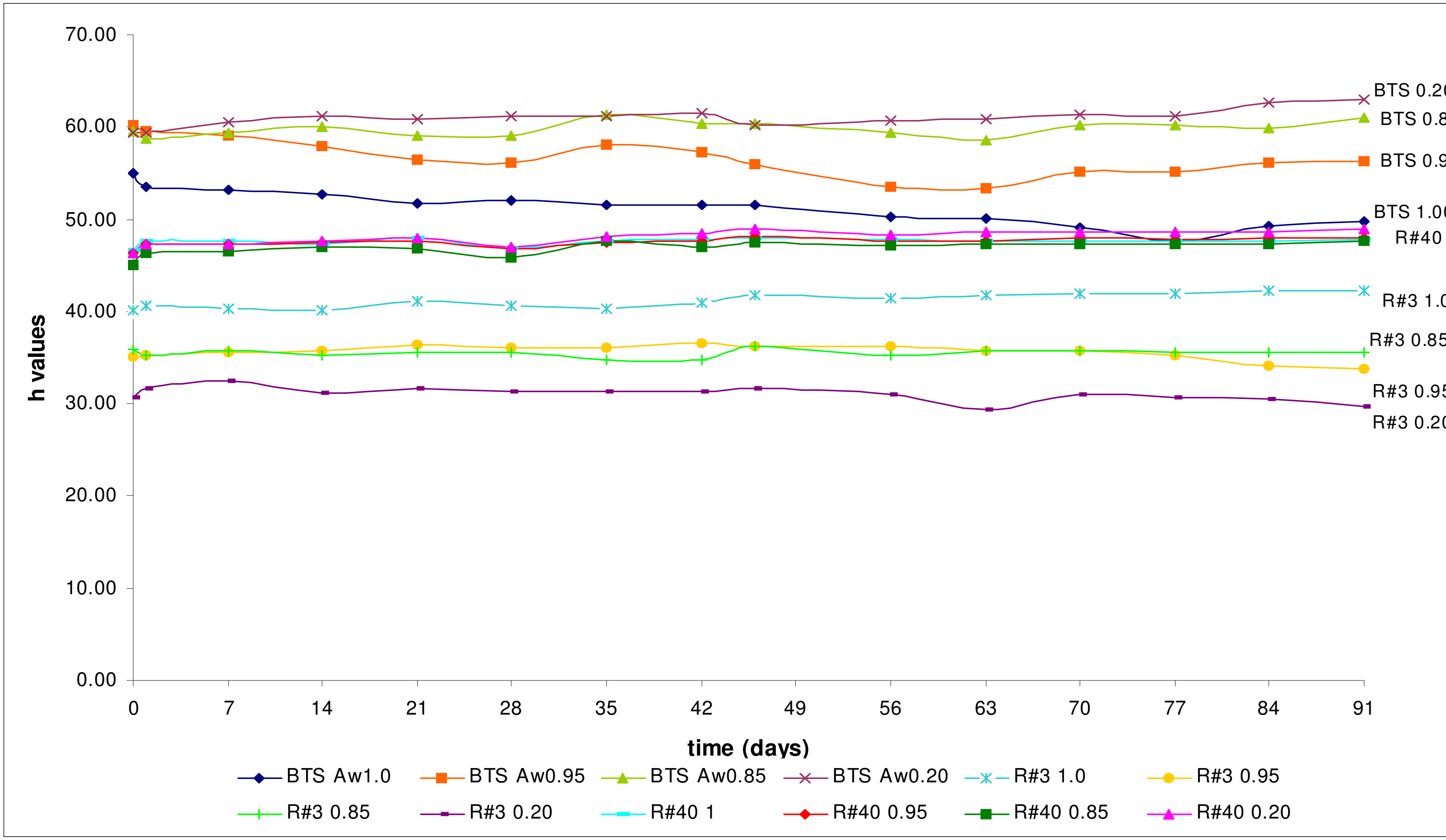


Fig. 39. Hue values of Black PI Tall sorghum bran extracts (BTS) and standard Red No.3 (R# 3) and Red No.40 (R#40) over time at different a_w. Concentration of 5mg of extract/mL of citric acid in aqueous ethanol at pH 2 and 25°C kept in the dark.

A major impediment to the use of anthocyanins as natural colorants is their instability in simple aqueous solutions and in complex food formulations (Gradinaru et al., 2003). The challenge of the water activity experiment was to dissolve the sorghum bran extract in aqueous solutions. When the sorghum bran extract was mixed with water, precipitation occurred affecting color measurements. Anthocyanin extracts have limited use in beverages which are hazy or cloudy, because the absorption into particulate matter can cause a blueing effect (Bridle and Timberlake, 1996). To get the water activity of 1.00, sorghum bran extracts were dissolved in distilled water. After time, precipitation occur affecting appearance. To obtain the water activity of 0.95 and 0.85, sucrose was used which eliminated precipitation. Thus, sucrose may protect sorghum 3-deoxyanthocyanins. Wrolstad reported that addition of sucrose to frozen strawberries had a protective effect on the anthocyanin pigments. Also, studies on strawberries showed that increasing the amount of sucrose by 20% increased anthocyanin stability (Vera de Rosso and Mercadante, 2007). The addition of 16% and 23% sucrose in a model beverage containing Amaranthus betacyanin showed that the anthocyanins were retained in large quantities. However, addition of 3 and 13% sucrose did not affect or slightly negatively affected the betacyanin stability (Cai and Corke, 1999).

Freeze-dried products containing anthocyanins with the lowest water activity have the highest stability. This was confirmed in another study with blue grape anthocyanins where the rate of degradation increased with water activity (Duangmal et al., 2007).

Water activity was plotted to see the similarities and/or differences in the color of the sorghum bran extracts and the synthetic colorants (Figs. 40, 41, 42, and 43). At high water activities, represented by 0.95, the hue and chroma values of BS and BTS were similar but more orange and dullest than synthetic colorants. Red #3 was the most red in color with hue similar to BTS and Red #40 had the most vivid color. Also, at low water activities, the hue and chroma values of BS and BTS were similar, but more orange and vivid. At 0.20, Red #40 had a red color. Red #3 was the most vivid and the color was between the sorghums and R#40.

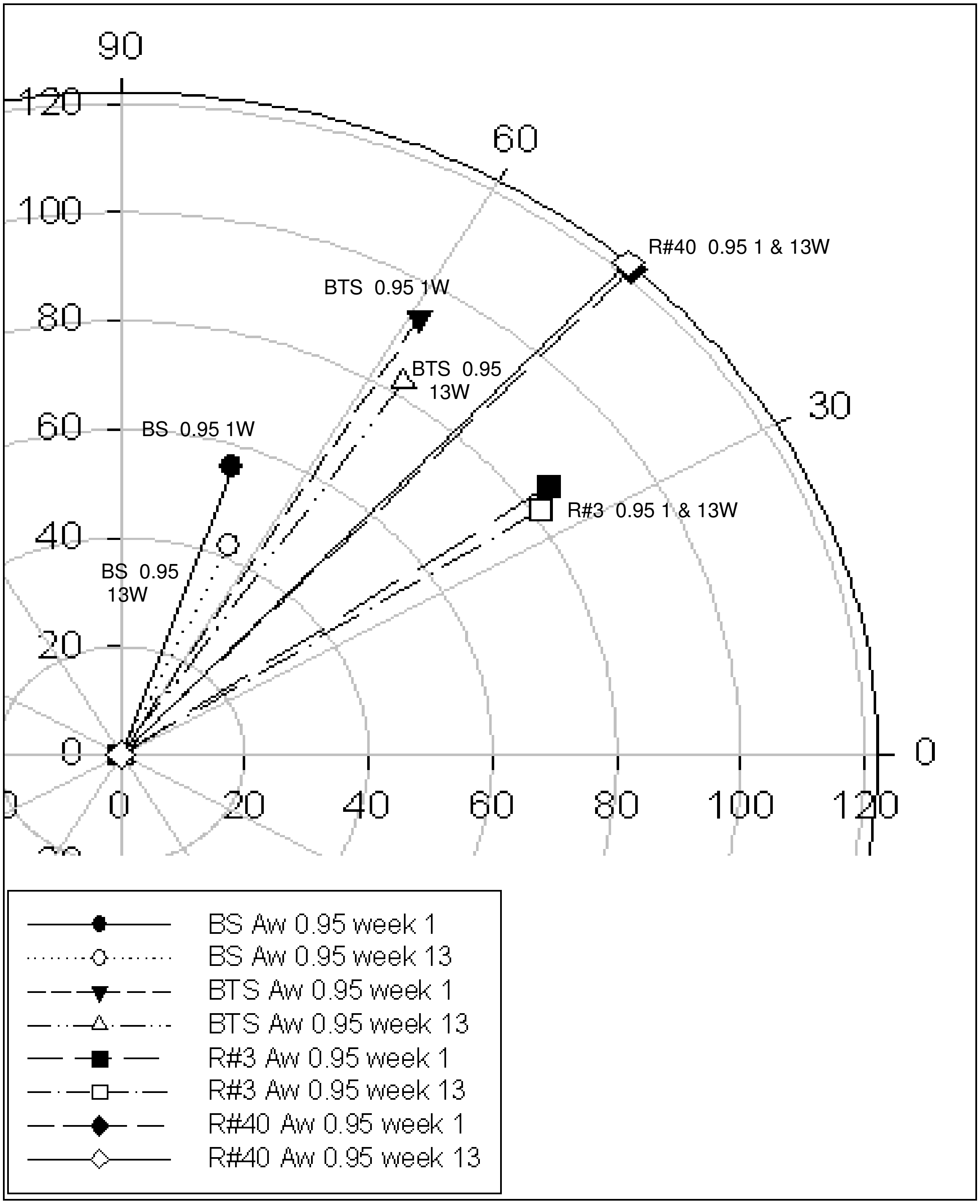


Fig. 40. Changes in color attributes of Tx430 Black (BS) and Black PI Tall (BTS) and standard Red No. 3(R#3) and Red No. 40(R#40) at Aw 0.95, after 1 and 13 weeks. Plot represents the chroma and the hue.

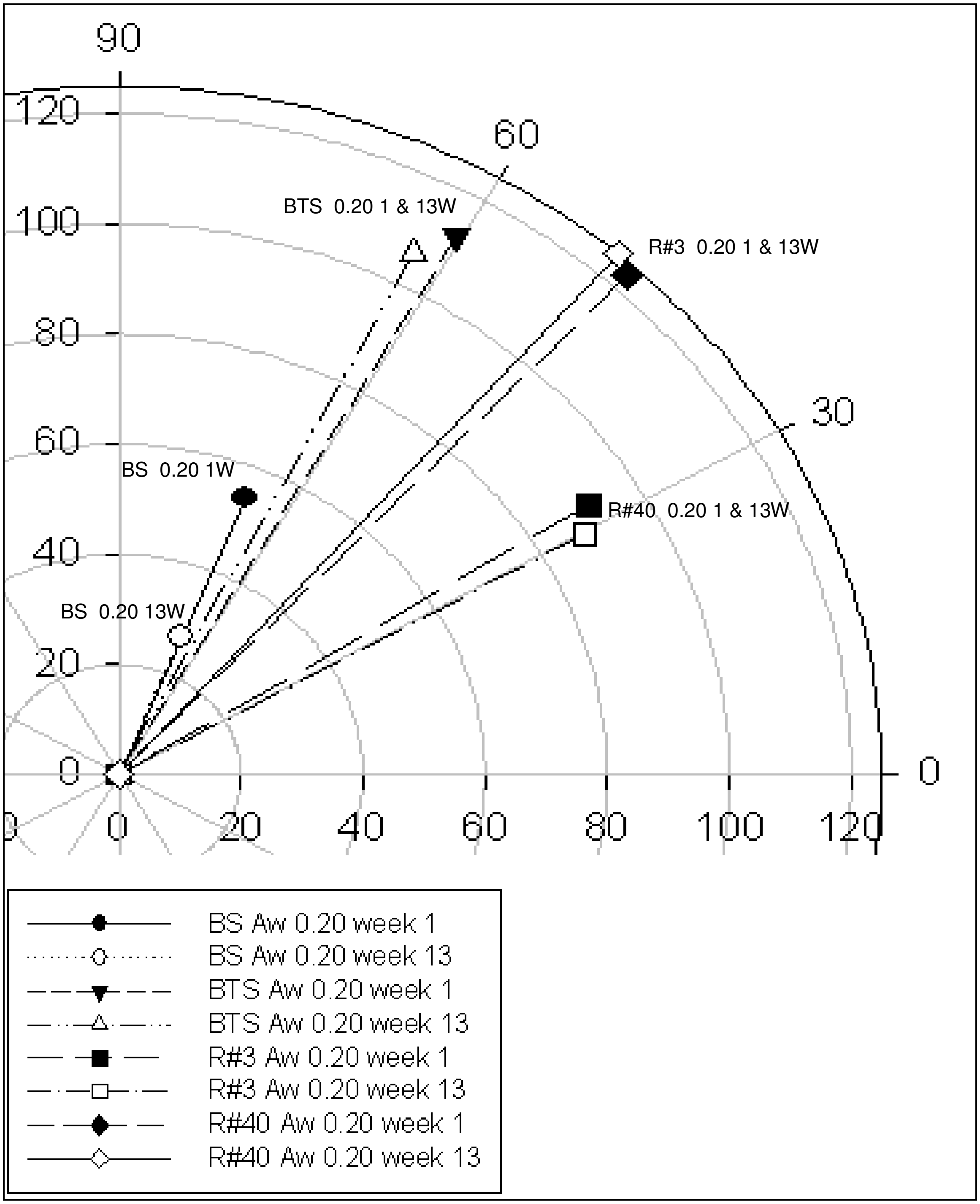


Fig. 41. Changes in color attributes of Tx430 Black (BS) and Black PI Tall (BTS) and standard Red No. 3(R#3) and Red No. 40(R#40) at Aw 0.20, after 1 and 13 weeks. Plot represents the chroma and the hue.

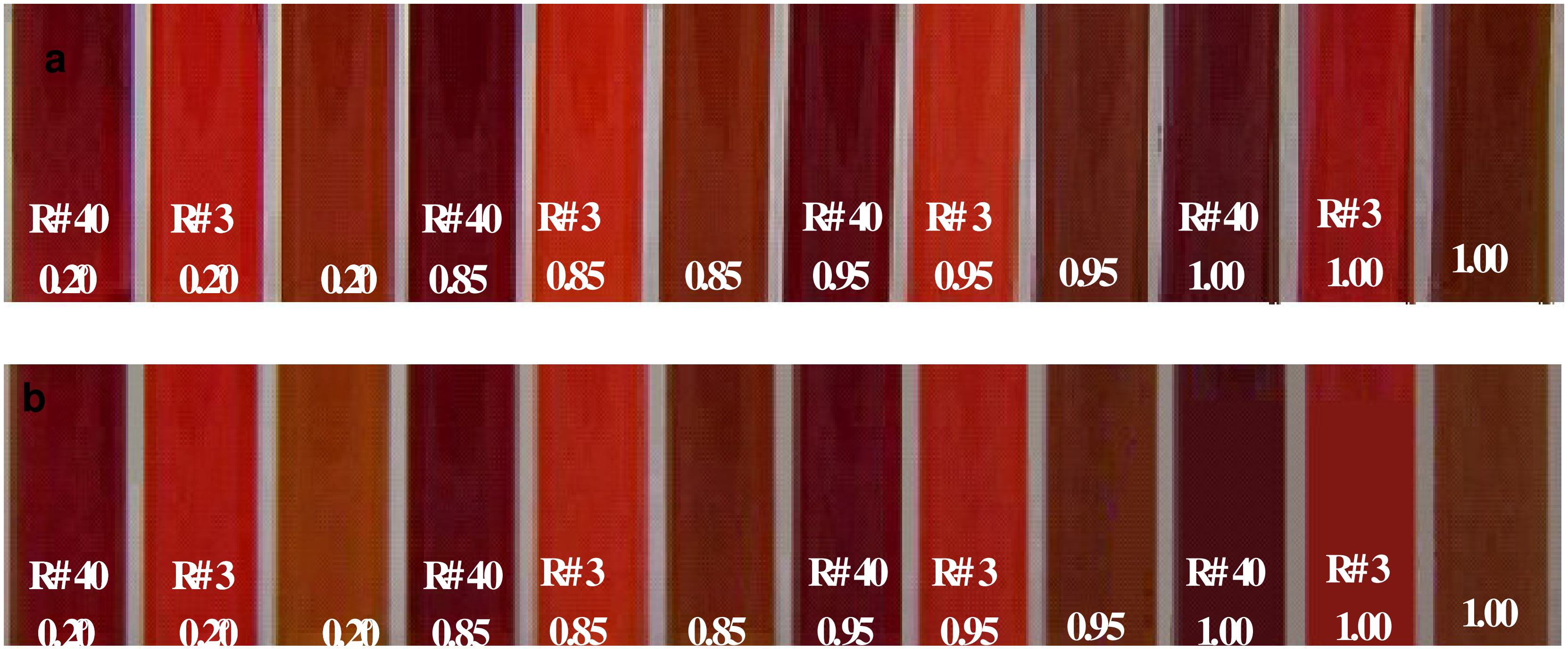


Fig. 42. Pictures of Tx430 Black sorghum bran extracted with 0.5% citric acid in 70% aqueous ethanol at different water activities. pH 2 at 25℃ after 1 week (a) and 13 weeks (b).

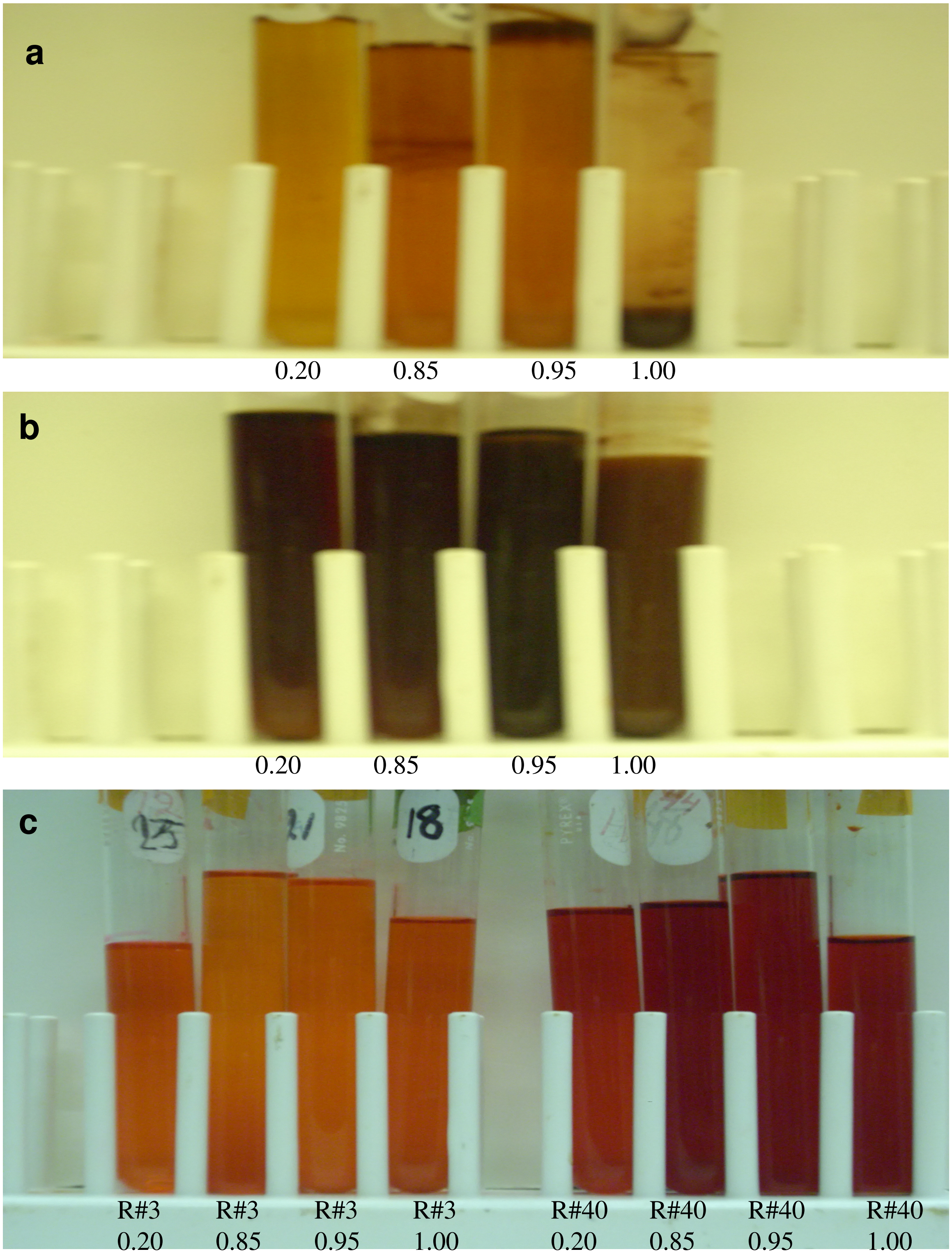


Fig. 43. Pictures of BS (a), BTS (b), and R#3 and R#40 (c) extracted with 0.5% citric acid in 70% aqueous ethanol at different water activities after 12 months. pH 2 at 25℃.

Concentration stability

Materials that possess high concentrations of anthocyanins are more suitable colorant sources. Anthocyanin pigments have greater stability when they are present at high concentrations. Also, increasing anthocyanins intensifies the color (Mazza and Miniati, 1993). Color stability was more affected by total anthocyanin concentration than by the quality of the pigment composition (Wrolstad, 2000).

Changes in visual color attributes of Tx430 Black sorghum bran extracts at different concentrations

Lightness of BS and Red #3 was measured and recorded for 13 weeks. Fig. 44 shows that as the concentration of BS increased, the lightness of the extracts decreased. The brightest sample was BS with a concentration of 0.5 mg/mL. At 1 mg/mL BS was comparable to Red #3 at concentration of 0.5 mg/mL after 7 weeks and with the standard at concentration of 1 mg/mL after 3 weeks of measurements. After 3 weeks, BS with concentration of 5 mg/mL did not change significantly. The L-values of the extracts were similar to the Red #3 with concentration of 10 mg/mL after 6 weeks; the L-values were between 50 and 53. After 1 week, L-values (39-44) of BS with concentration of 10mg/mL were comparable with Red #3 with concentration of 20 mg/mL. The darkest samples were BS with concentration of 40 mg/mL and 80 mg/mL. After 7 weeks, L-values from BS with concentration of 80 mg/mL were almost undetectable by the colorimeter.

From all BS, the one with concentration of 5 mg/mL had the most vivid color with values around 100 during all the 13 weeks (Fig. 45). C-values of BS with concentrations of 1 mg/mL and 20 mg/mL were statistically similar ($P<0.05$) over time after 1 and 3 weeks, respectively. Chroma values of BS with concentration of 0.5 mg/mL were constant after 3 weeks and the extracts with concentration of 40 mg/mL after 4 weeks. The low C-values (between 37 and 47) indicated that these samples did not have a vivid color. The C-values of the BS with concentration of 80 mg/mL were constant but the values below 20 indicated that from all the samples, the extracts at this concentration had the dullest color. Red #3 at all concentration showed more vivid colors than BS with the exception of concentration of 5 mg/mL, which showed high C-values of approximately 110. Because of these high values, the BS at this concentration can be comparable with Red #3 with concentrations of 20 mg/mL and 40 mg/mL. The most unstable synthetic colorant sample was at concentration of 0.5 mg/mL; the C-values started at 119 and by the end of the experiment, the values increased to around 132. Red #3 at concentrations of 5 and 10 mg/mL was the most stable with C-values of 125.

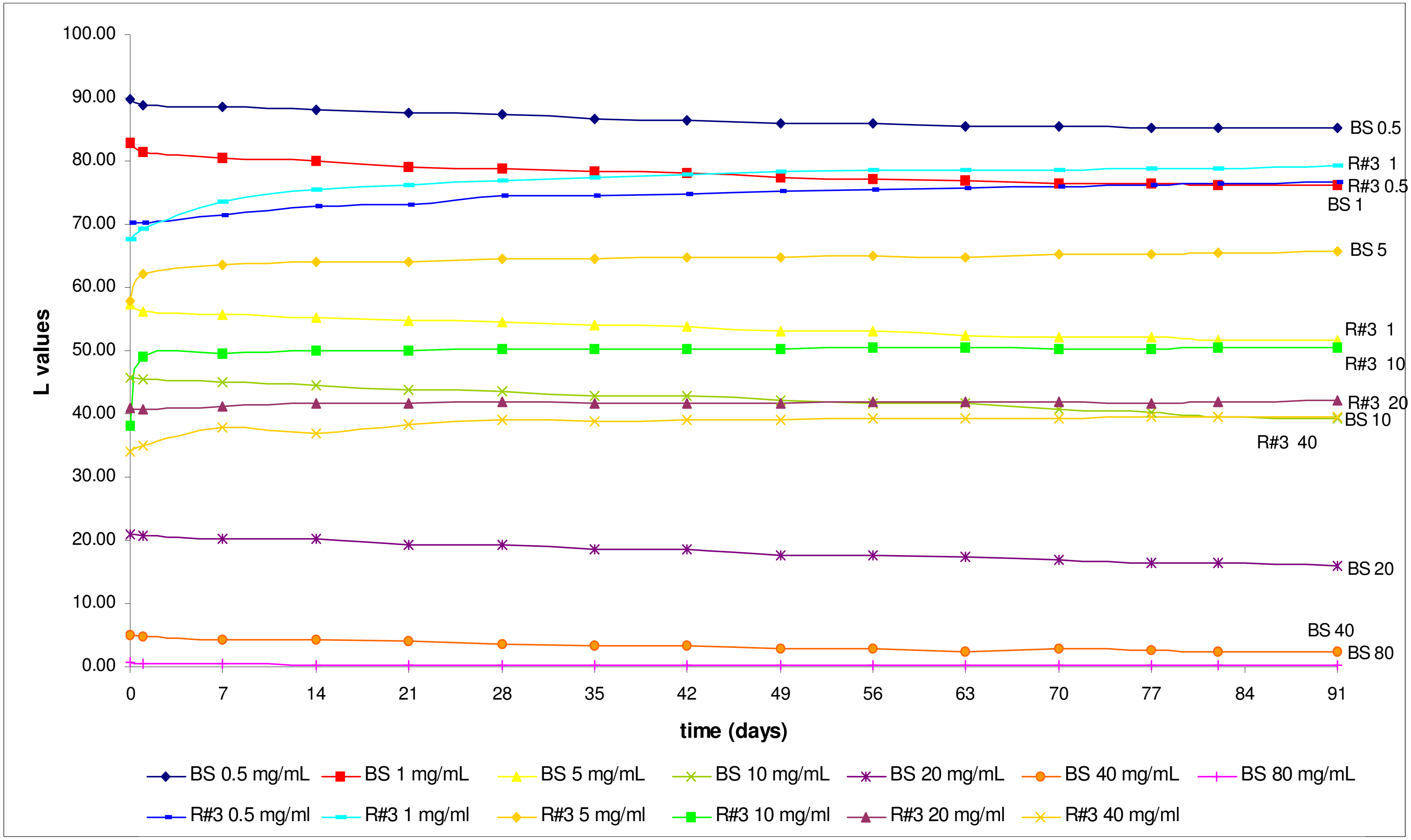


Fig. 44. Lightness values of Tx430 Black sorghum bran extracts (BS) and standard Red No.3 (R# 3) over time at different concentrations. Extracted with citric acid in aqueous ethanol at pH 2. Kept in the dark at 25℃.

As the concentration of the BS increased, hue value decreased and the color changed from yellow orange to red (Fig. 46). The highest hue values were for BS with concentrations of 0.5 and 1 mg/mL with values of around 70 that remained constant through time. These two samples are comparable to Red #3 with concentrations of 0.5 mg/mL and 1 mg/mL; the C-values were similar to the BS after 6 weeks of measurements. BS at 5 mg/mL is comparable to Red #3 with concentration of 5 mg/mL after 3 weeks. After 5 weeks, hue values of BS at 10 mg/mL (around the orange region) showed similitude with the Red #3 at the same concentration. Red #3 with concentration of 20 mg/mL and 40 mg/mL was constant tover time; the hue values were located in the orange region (around 40). Between these samples, no significant differences were found. The hue values of BS at a concentration of 20 mg/mL showed a small decrease, which means that with time the color of the extracts became more red. BS at a concentration of 80 mg/mL had the lowest hue values. At that concentration, BS had a deep red color.

Changes in visual color attributes of Black PI Tall sorghum bran extracts at different concentrations

The BTS followed the same trend that the BS: if the concentration of the extracts increased the lightness of the extracts decreased (Fig. 47). The brightest sample was the BTS at a concentration of 0.1 mg/mL with values above 90. This was followed by the BTS with a concentration of 0.5 mg/mL. The L-values of this extract (around 73) were constant over time. After 1 week, Red #3 with concentrations of 0.1, 0.5 and 1.0 mg/mL showed similar L-values to BTS at

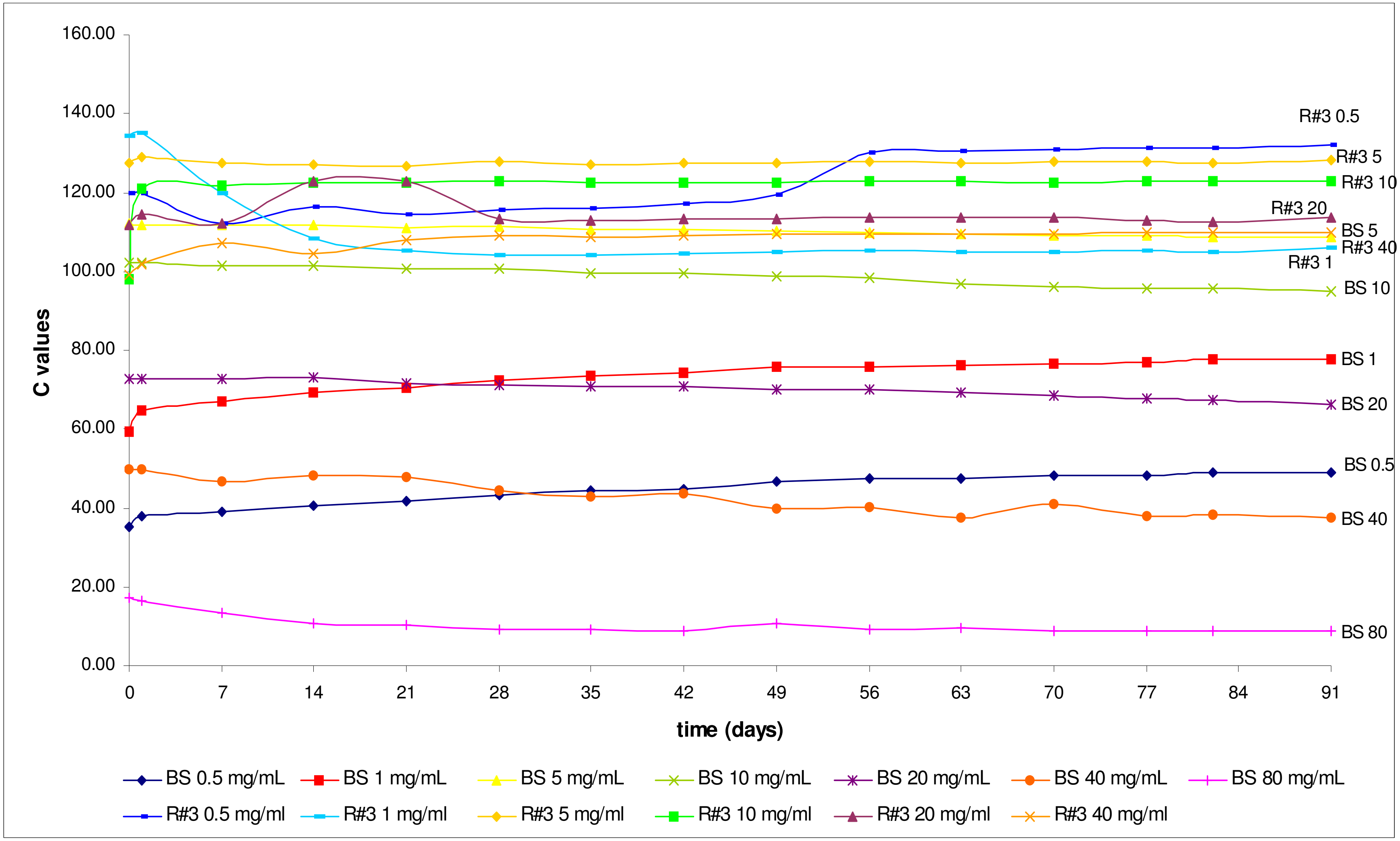


Fig. 45. Chroma values of Tx430 Black sorghum bran extracts (BS) and standard Red No.3 (R# 3) over time at different concentrations. Extracted with citric acid in aqueous ethanol at pH 2. Kept in the dark at 25°C.

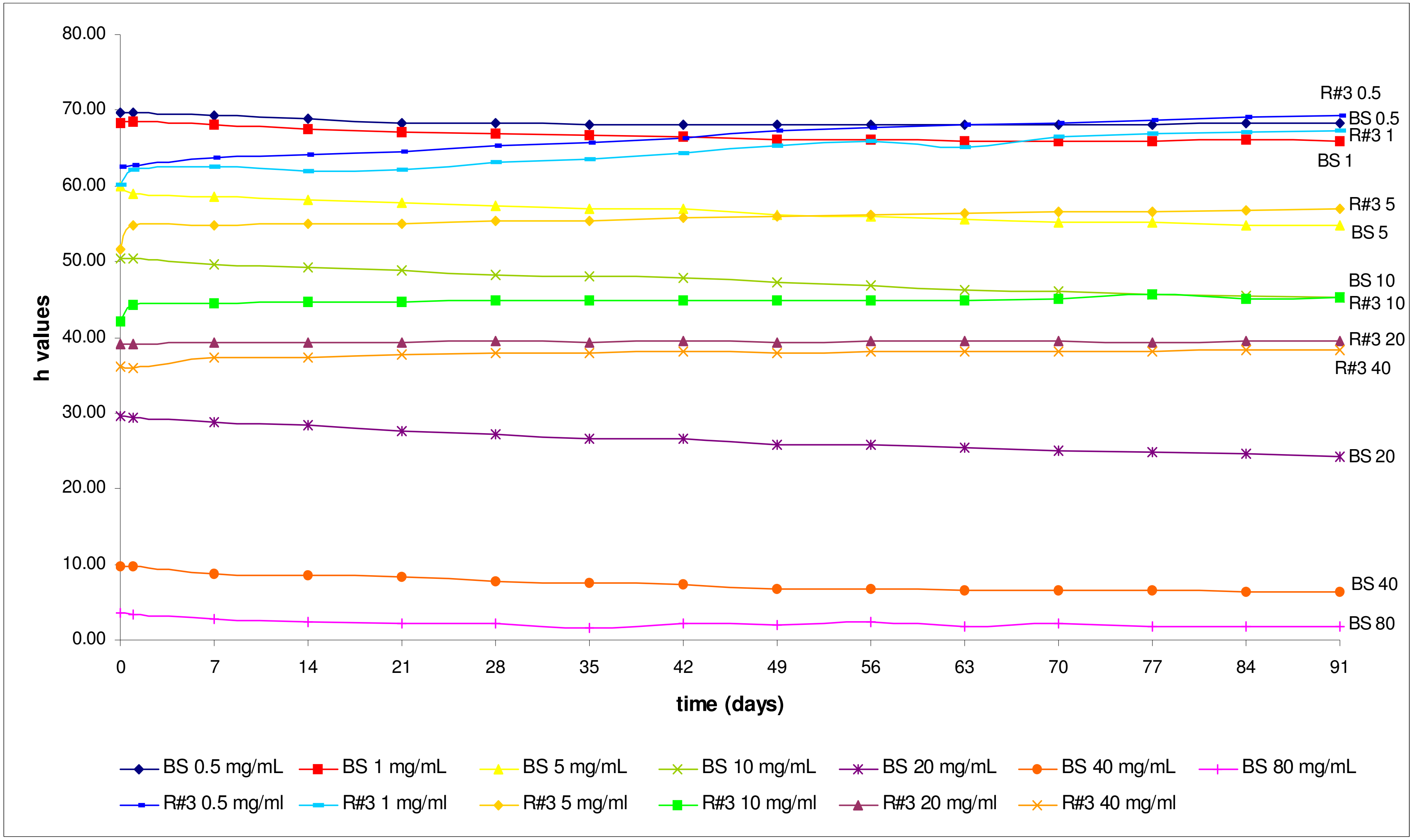


Fig. 46. Hue values of Tx430 Black sorghum bran extracts (BS) and standard Red No.3 (R# 3) over time at different concentrations. Extracted with citric acid in aqueous ethanol at pH 2. Kept them in the dark at 25℃.

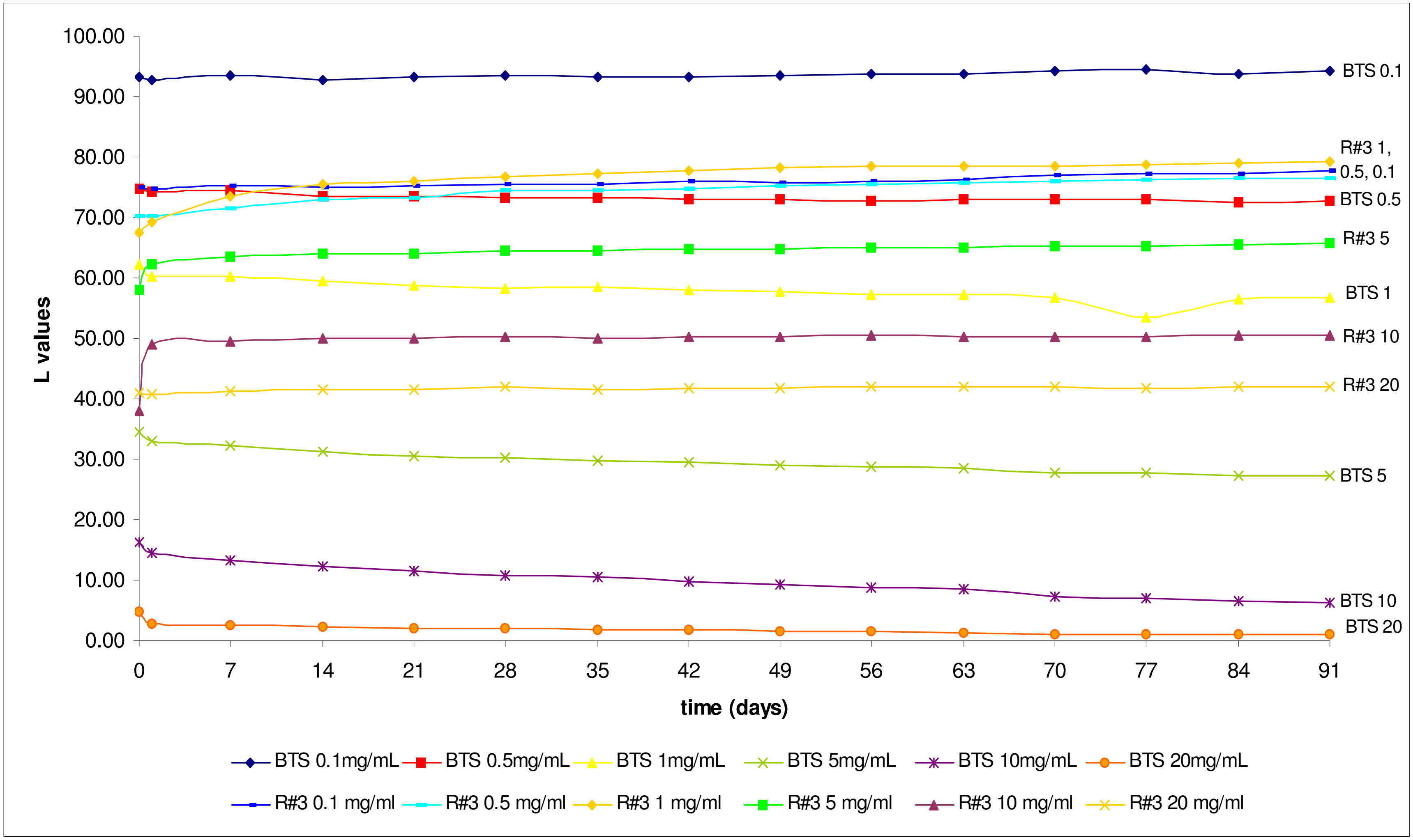


Fig. 47. Lightness values of Black Pl Tall sorghum bran extracts (BTS) and standard Red No.3 (R# 3) over time at different concentrations. Extracted with citric acid in aqueous ethanol at pH 2. Kept in the dark at 25℃.

0.5 mg/mL. Hence, this extract is a good substitute for the standard at the concentrations mentioned before. At a concentration of 1 mg/mL, BTS also showed good stability over time. After 4 weeks, BTS with a concentration of 5 mg/mL were stable. BTS at concentrations of 10 and 20 mg/mL were stable after 8 and 6 weeks, respectively. Red #3 with concentrations of 5, 10, and 20 mg/mL was brighter than BTS with concentration of 5, 10, and 20 mg/mL. With time, L-values of BTS with concentration of 10 mg/mL decreased, becoming less bright. The lowest L-values was for the 20 mg/mL extract, with values nearly zero (almost dark).

The dullest BTS was the one with a concentration of 0.1 mg/mL, with constant values of around 20 (Fig. 48). BTS with concentration of 0.5 mg/mL also showed had stable C-values over time. After 4 weeks, BTS with concentrations of 1 and 5 mg/mL were constant. The difference was that BTS with concentration of 1 mg/mL showed vivid color with values equal to Red #3 at concentration of 20 mg/mL after 3 weeks. BTS with concentration of 5 mg/mL showed lower C-values (around 85) and any concentration of Red #3 was comparable with it. The C-values of BTS with concentration of 20 mg/mL decreased significantly over time and just after 10 weeks, the C-values were constant. Red #3 had the highest C-values. The most stable C-values over time were Red #3 at concentrations of 5 mg/mL (around 127) and 10 mg/mL (around 122). Red #3 at 0.1 mg/mL was unstable and the C-values increased from 103 to near 127. Red #3 with concentration of 0.5 mg/mL was also unstable since their

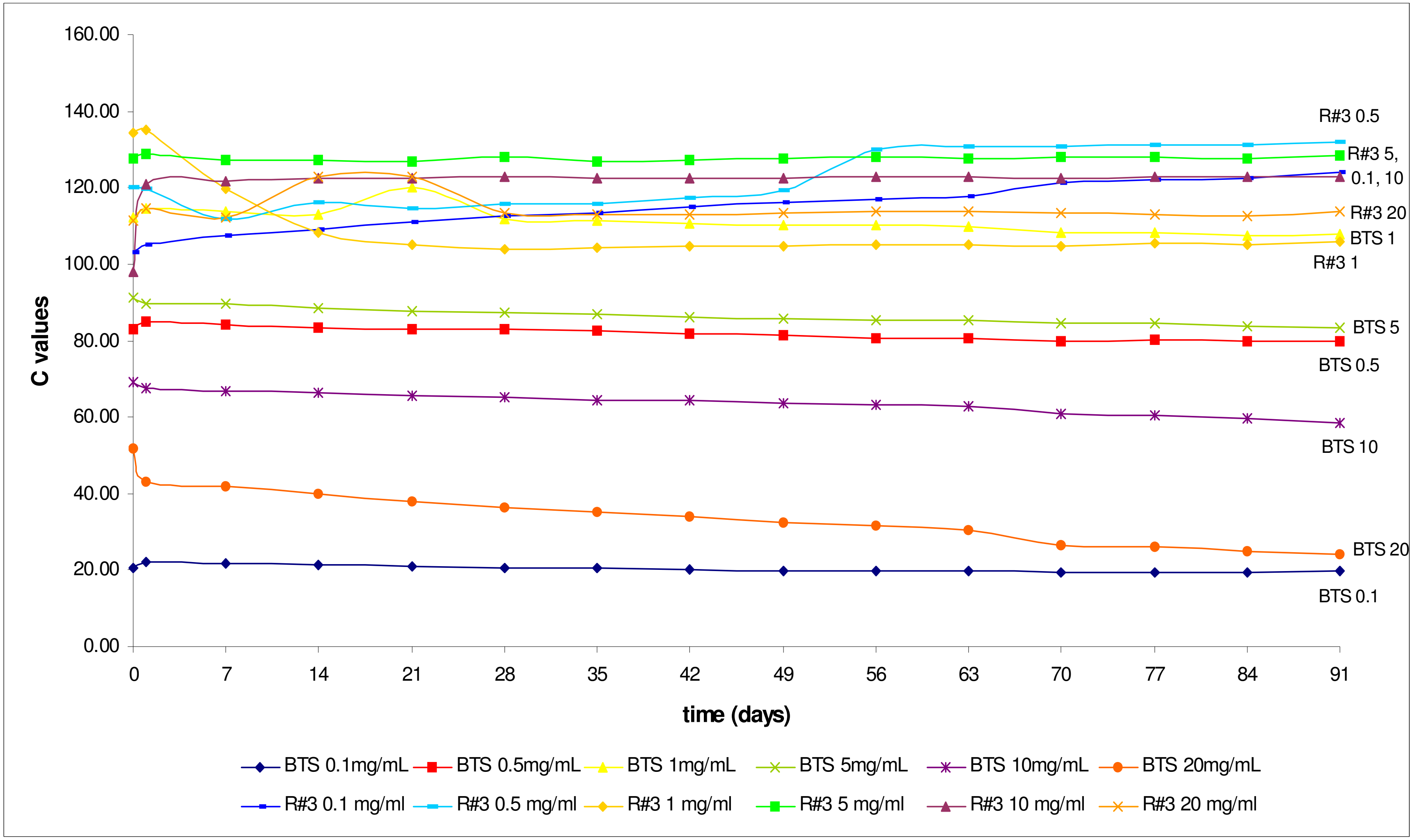


Fig. 48. Chroma values of Black PI Tall sorghum bran extracts (BTS) and standard Red No.3 (R# 3) over time at different concentrations. Extracted with citric acid in aqueous ethanol at pH 2. Kept in the dark at 25℃.

values changed from 115 to 132. Red #3 with concentrations of 1 and 20 mg/mL were stable after 3 and 4 weeks, respectively.

As the concentration of BTS and Red #3 increased the color became more red (hue values decreased) (Fig. 49). Sorghum bran extract with a concentration of 0.1 mg/mL had the highest hue values; the extract was stable until week 10 when the extracts moved more to the yellow region. At that concentration, BTS was comparable in color to Red #3 with a concentration of 0.5 mg/mL. Hue values (yellow-orange color) of BTS with a concentration of 0.5 mg/mL did not change over time. The color was very similar to that of Red #3 with concentration of 1 mg/mL. After 6 weeks, the hue values of BTS with a concentration of 1 mg/mL were similar to the hue values of Red #3 with a concentration of 5 mg/mL; under these conditions BTS could replace the synthetic colorant. At all concentrations, Red #3 had hue values between the orange and orange-yellow range (above 40). BTS at 5 mg/mL had close values to Red #3 at concentration of 20mg/mL. The hue values of BTS with a concentration of 10 mg/mL decreased. BTS with concentration of 20 mg/mL had the lowest hue values showing red color.

In a study where anthocyanins from acerola were added to isotonic systems, high anthocyanin concentration decreased degradation (Vera de Rosso and Mercadante, 2007). The data presented before showed that, at any concentration, sorghum bran extracts were stable. However, lightness, chroma, and hue changed with concentration.

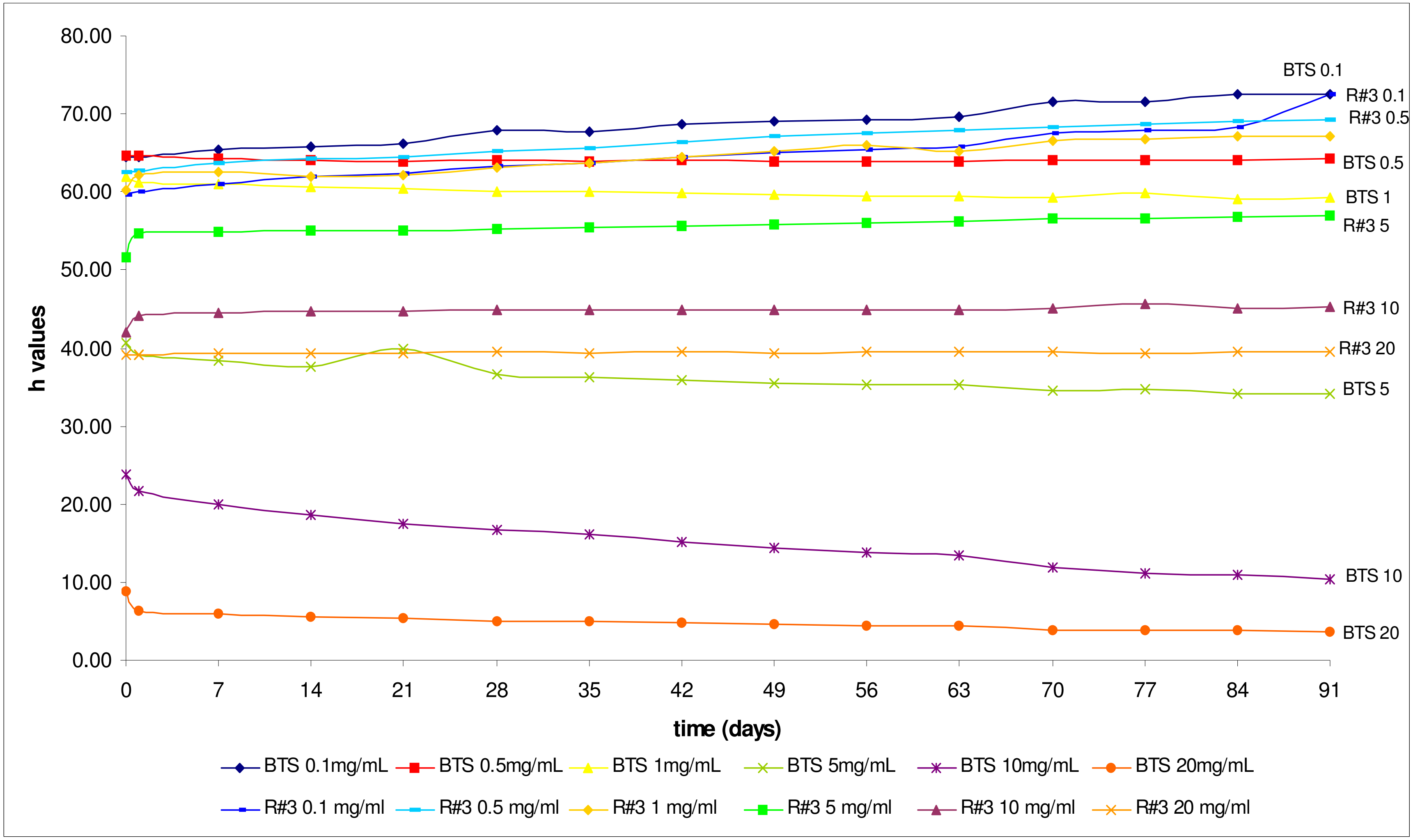


Fig. 49. Hue values of Black PI Tall sorghum bran extracts (BTS) and standard Red No.3 (R# 3) over time at different concentrations. Extracted with citric acid in aqueous ethanol at pH 2. Kept them in the dark at 25℃.

Giusti and Wrolsatd (1996) added a radish anthocyanin extract to maraschino cherries and found that the chroma values decreased at higher rates in samples with lower anthocyanins concentrations. The hue values also showed higher reduction over a shorter period of time in samples with low anthocyanin concentration. Meanwhile, reduction in hue values of samples with high anthocyanin concentration was obtained after a longer period of time. Decreases in the C-values of isotonic drinks with anthocyanins from acai and acerola showed that the red color intensity decreased during storage. These changes also were accompanied with changes in color from red to yellow color (Vera de Rosso and Mercadante, 2007). Contrary to those studies, the 3-deoxyanthocyanins from both black BS and BTS remained very stable in lightness, chroma and hue values over time.

Comparisons of the color changes at different concentrations were plotted in Figs. 50, 51, 52, 53, and 54. At low concentration, the hue values of the black sorghum bran extracts were comparable to Red #3. The main difference was the chroma values; the C-values of Red #3 were the highest and those of the BS were the lowest. At concentration of 5 mg/mL, BS was comparable to Red #3. The same behavior was shown at a concentration of 10 mg/mL, where BS was comparable to Red #3. As concentration increased the color of BTS moves to the red region.

The previous results proved the BS and BTS can be used instead of the Red #3 and Red #40 when they are used at the right pH, temperature, water activity, and/or concentration.

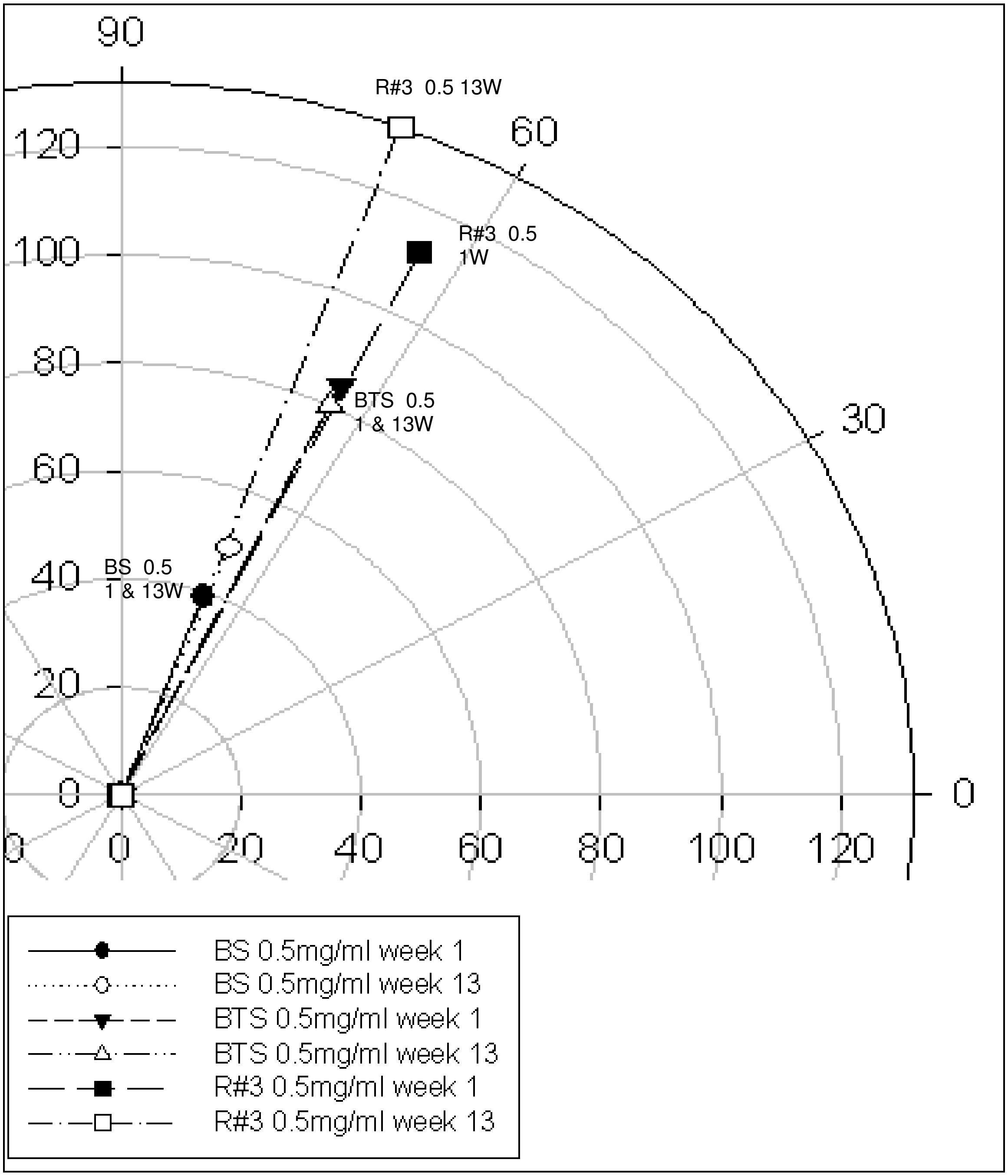


Fig. 50. Changes in color attributes of Tx430 Black (BS) and Black PI Tall (BTS) and standard Red No. 3(R#3) and Red No. 40(R#40) at 0.5 mg/mL, after 1 and 13 weeks. Plot represents the chroma and the hue.

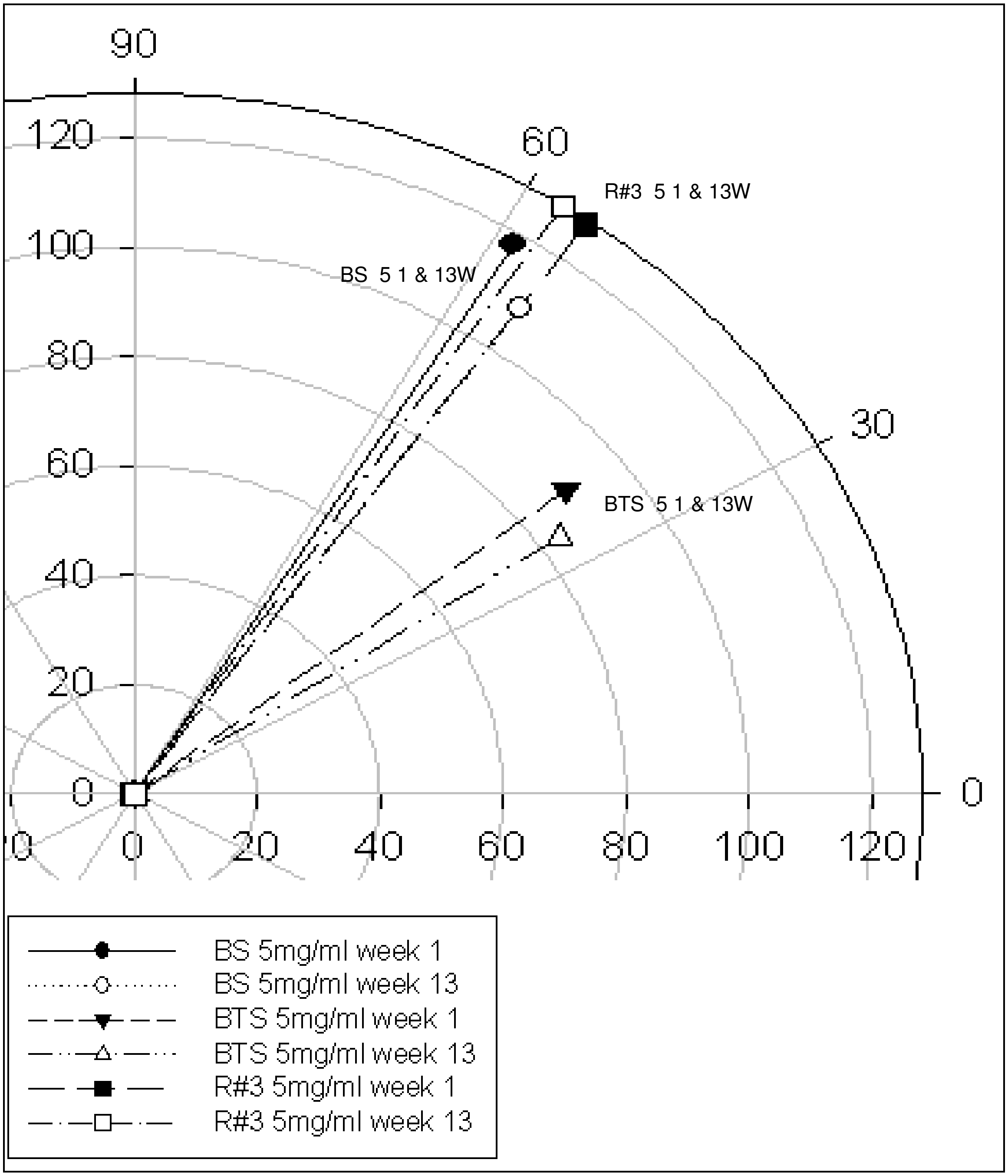


Fig. 51. Changes in color attributes of Tx430 Black (BS) and Black PI Tall (BTS) and standard Red No. 3(R#3) and Red No. 40(R#40) at 5 mg/mL, after 1 and 13 weeks. Plot represents the chroma and the hue.

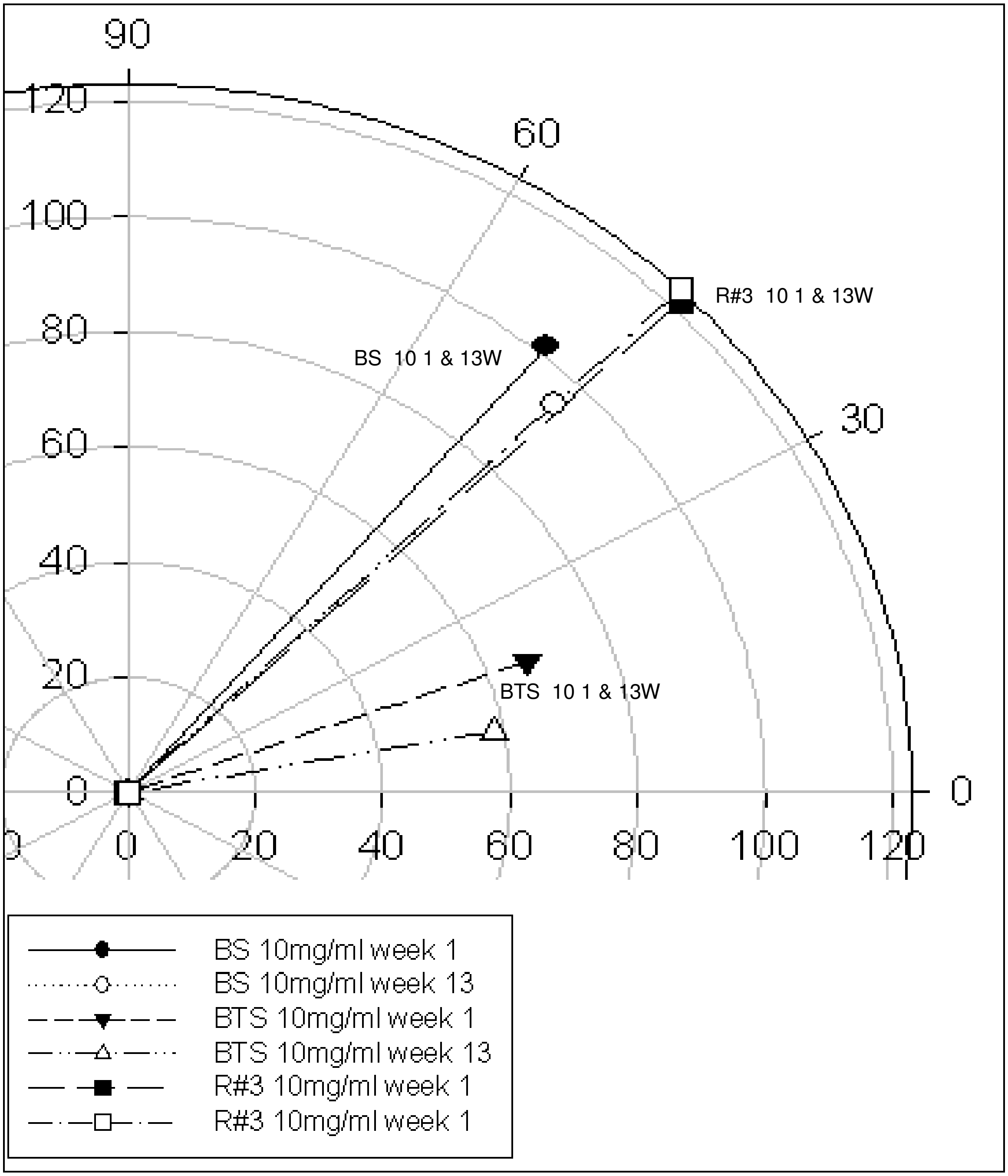


Fig. 52. Changes in color attributes of Tx430 Black (BS) and Black PI Tall (BTS) and standard Red No. 3(R#3) and Red No. 40(R#40) at 10 mg/mL, after 1 and 13 weeks. Plot represents the chroma and the hue.

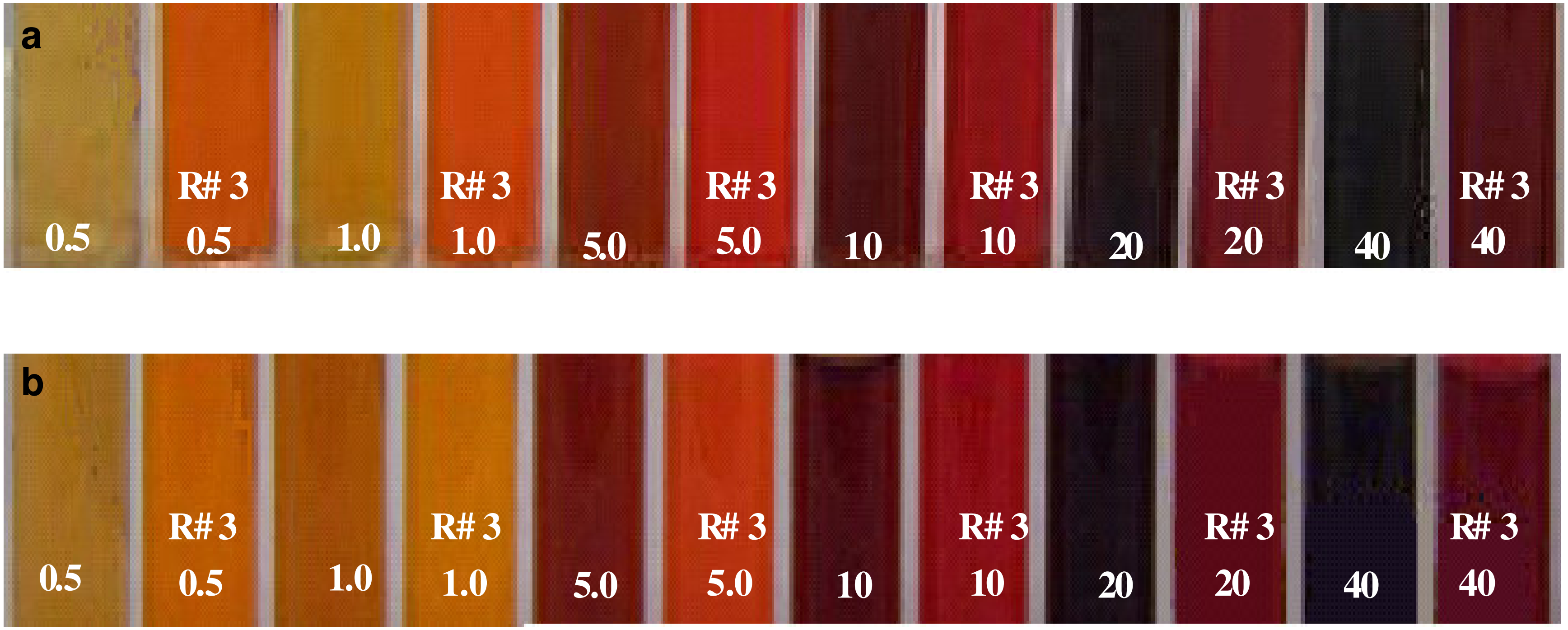


Fig. 53. Pictures of Tx430 Black sorghum bran extracted with 0.5% citric acid in 70% aqueous ethanol at different concentrations. pH 2 at 25℃ after 1 week (a) and 13 weeks (b).

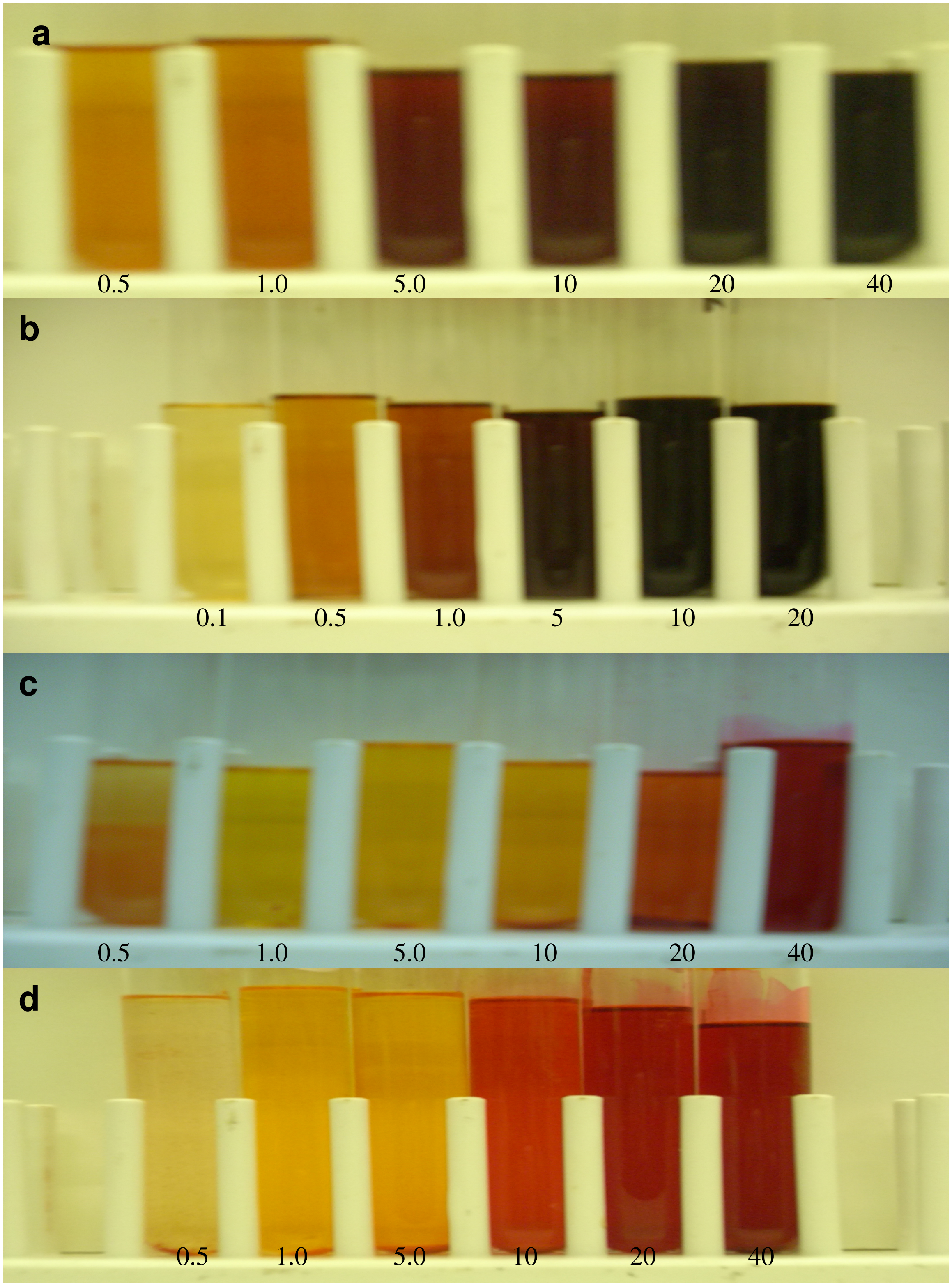


Fig. 54. Pictures of BS (a), BTS (b), and R#3 (c) and R#40 (d) extracted with 0.5% citric acid in 70% aq. ethanol at different Aw after 12 months. pH 2 at 25℃.

Comparison of Tx430 Black and Black PI Tall sorghum bran extracts with Red No. 3

Comparison of the color parameters lightness, chroma, and hue at the beginning and at the end of the experiment were done to see the differences or similarities between the samples studied.

The behavior of the three color parameters studied in BS followed the same trend at week one and at week 13 (Fig. 55). From concentration 0.5 mg/mL to 5 mg/mL, chroma values increase drastically (more than 70 units) but after this concentration the values started to decrease until they reach values around 10. This decrease in saturation may be due to the formation of colorless carbinol. Lightness and hue values decreased as the concentration of the sorghum bran extracts increased, which meant that as the concentration increased, the extracts became darker and the color moved to the red color region. This is good because you can target the desirable color in food by changing the concentration of the sorghum extract.

Like the BS, the behavior of the L, C, and h values in BTS followed the same trend at the beginning and at the end of the experiment (Fig. 56). The values of the parameters measured were constant over time so no significant differences were found between week 1 and week 13. From concentration of 0.1 mg/mL to 1 mg/mL, the chroma values increased by almost 100 units and then the values decreased. The difference between BS and BTS was that BTS started with lower C-values and by the end of the experiment the values were higher than those of BS. As the concentration of BTS increased, the lightness decreased, which meant less bright extracts. Also the hue values decreased, which changed the color from orange to red.

Very different trends were shown by Red #3 at week 1 and week 13 (Fig. 57). The C-values at the beginning of the experiment were constant at the different concentrations. But after 13 weeks, the C-values from concentration 0.5 mg/mL to 1 mg/mL decreased and at 5 mg/mL increased again. The lightness and the hue angle followed similar behavior at the start and end of the experiment. As the concentration of Red #3 increased the samples were less bright and the color became more red. During the 13 weeks, the L- and h- values were stable so no differences between week 1 and week 13 were found.

The slight decrease in C-values of the sorghum bran extracts and the synthetic colorant had a different behavior than common anthocyanins. C-values of fruits and vegetables increase as the concentration increase. This suggests that in other to have the same color is important to choose the right concentration.

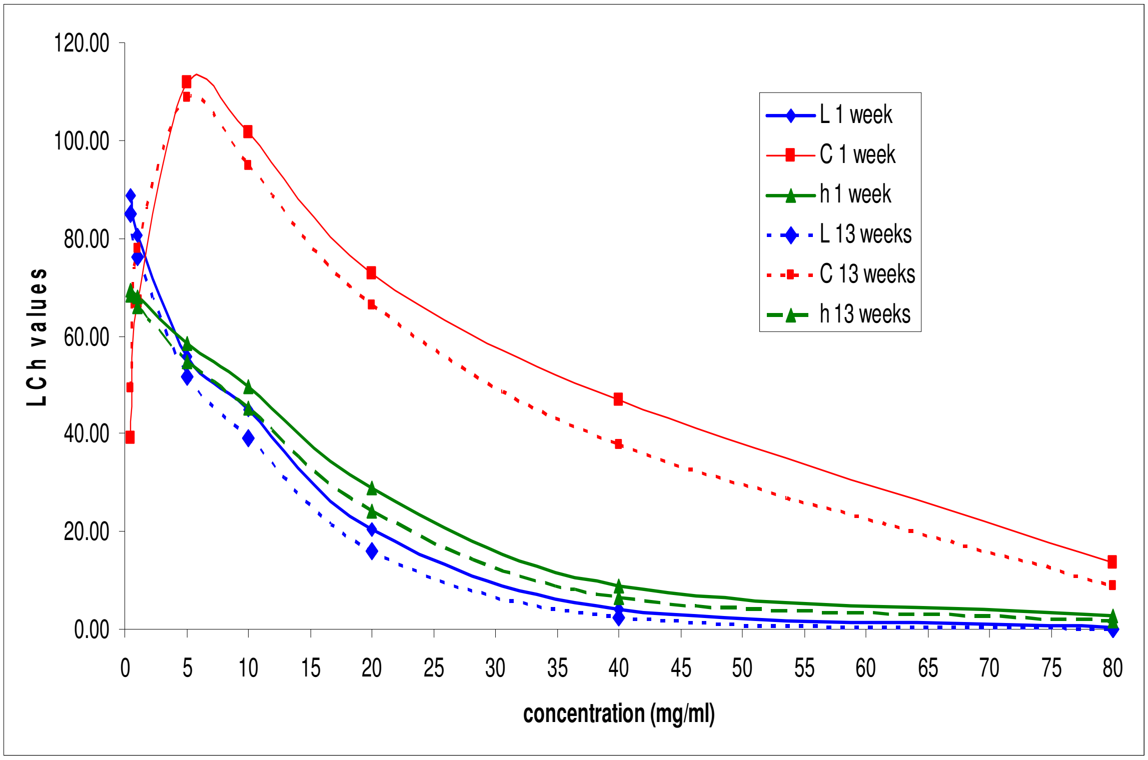


Fig. 55. Change in the color parameters values lightness (L), chroma (C), and hue (h) for Tx430 Black sorghum bran (BS) extracted with citric acid in aqueous ethanol at pH 2 kept in the dark at 25°C after 1 and 13 week.

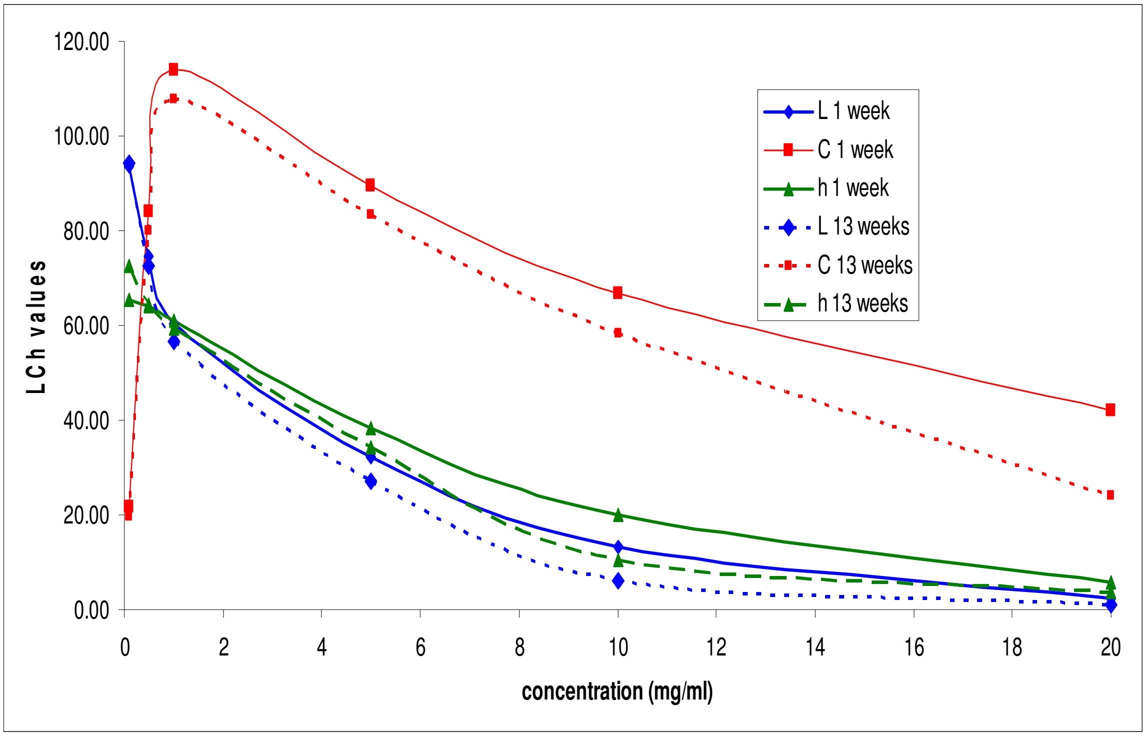


Fig. 56. Change in the color parameters values lightness (L), chroma (C), and hue (h) for Black PI Tall sorghum bran (BTS) extracted with citric acid in aqueous ethanol at pH 2 kept in the dark at 25℃ after 1 and 13 week.

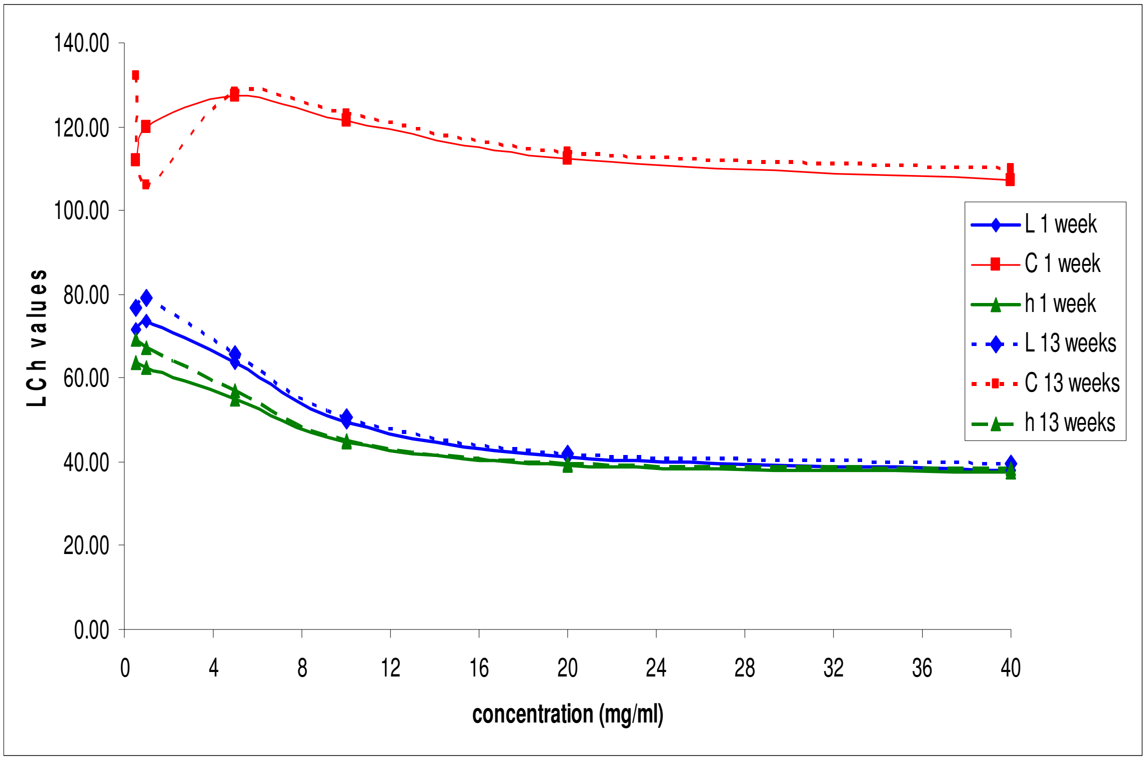


Fig. 57. Change in the color parameters values lightness (L), chroma (C), and hue (h) for synthetic colorant Red No. 3 (R#3) dissolved with citric acid in aqueous ethanol at pH 2 kept in the dark at 25℃ after 1 and 13 week.

CHAPTER VII

SUMMARY AND CONCLUSIONS

The differences in absorbance and visual color parameters between the Tx430 Black (BS) and Black PI Tall (BTS) sorghum bran extracts at different pHs, temperatures, water activities, and concentrations may be due to the differences in the compounds of each sorghum; tannins in Black PI Tall may contribute to the color stability.

Under high temperature conditions (98°C for two hours) black sorghum extracts were stable, especially at pH above 3.

At different pHs, color attributes of black sorghum extracts showed good stability. BTS showed better stability over time than BS. In general, BS showed hues (orange) similar to Red #3, and BTS had similar hue (orange-red) values to Red #40. Chroma values of the synthetic colorants were higher (vivid) than black sorghum bran extracts. By changing pH, color of black sorghum brans could be optimized to give different lightness, chroma, and hue to be used in specific food systems.

Lightness, chroma and hue values of the black sorghum bran extracts were stable at all temperatures studied with the exception of those samples that were heated at 50ºC. The instability of the extracts at high temperature can be a consequence of the degradation of anthocyanins which forms brown products. BTS was comparable to Red #40; hue values (orange color) were similar at all temperatures for 13 weeks. Red #3 showed more variation with poor stability.

Color parameters for black sorghum extracts showed different stabilities at different water activities. In general, BS showed better stability at water activities of 0.95 and 0.85. BTS showed good stability, especially at low water activities. As the water activity increased the values of lightness, chroma and hue decreased. Red #3 and Red #40 showed good stability at different water activities. At low water activities BTS can be comparable to Red #40. The stability was improved by the addition of sucrose which also helps to reduce water activity.

At different concentrations, the color parameters of black sorghum bran were very stable. As the concentration increased, the values of lightness, chroma, and hue decreased (become more red). In general, at low concentrations the BS and BTS had hue values similar to Red #3. As the concentration increased, the BTS became more red thus they were no longer comparable to the standard colorant. Vividness of color from black sorghum bran extracts could be tailored to different food systems by changing concentration.

Data obtained with colorimeter showed differences in the color parameters among samples. These differences might not be perceived by human eye; therefore a sensory analysis could be used to get the human perception.

In conclusion, the results from this study showed that the black sorghum bran with tannins (Black PI Tall) and without tannins (TX430 Black) have great potential for use in the food industry as colorants. They may replace Red #3 and Red #40. Further investigation comparing black sorghum bran extracts with commercial anthocyanins like red cabbage is needed.

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APPENDIX A

BLACK SORGHUM BRAN STATISTICS

Table VI Statistical analysis of lightness of sorghum TX430 Black at different pHs. ($p<0.05$)

	pH 1	SD	pH 2	SD	pH3	SD	pH 4	SD	pH 5	SD	pH 6	SD
0 day	53.93^{a}	4.53	52.78^{a}	0.26	52.33^{a}	0.35	44.79^{a}	0.52	35.59^{a}	1.94	37.08^{a}	1.52
1 day	$55.87^{a,b}$	1.13	53.15^{a}	0.34	52.99^{a}	0.56	46.7^{a}	0.48	41.93^{b}	1.35	40.07^{a}	2.17
1 week	67.517^{c}	0.76	65.173^{b}	0.27	65.25^{b}	0.38	62.86^{b}	0.40	61.74^{c}	0.31	$56.28^{a,b}$	1.47
2 week	66.79^{c}	0.97	65.0033^{b}	0.29	65.04^{b}	0.29	62.76^{b}	0.42	61.46^{c}	0.31	$56.00^{a,b}$	0.34
3 week	65.98^{c}	1.54	65.033^{b}	0.45	65.33^{b}	0.29	63.06^{b}	0.53	61.45^{c}	0.62	$56.20^{a,b}$	0.37
4 week	65.44^{c}	1.95	64.56^{b}	0.60	64.70^{b}	0.33	62.79^{b}	0.52	60.65^{c}	0.78	$55.73^{a,b}$	0.86
5 week	$64.92^{b,c}$	2.14	64.33^{b}	0.74	64.73^{b}	0.28	62.61^{b}	0.64	60.08^{c}	0.72	$55.27^{a,b}$	1.61
6 week	$64.42^{b,c}$	2.39	64.277^{b}	0.87	64.68^{b}	0.52	62.76^{b}	0.73	60.11^{c}	1.26	$55.50^{a,b}$	2.17
7 week	$64.17^{b,c}$	2.22	64.32^{b}	1.38	64.41^{b}	0.64	62.71^{b}	0.68	59.66^{c}	1.60	$55.34^{a,b}$	2.68
8 week	$65.15^{b,c}$	0.01	64.39^{b}	0.13	64.14^{b}	0.79	62.60^{b}	0.84	59.24^{c}	1.91	$55.21^{a,b}$	3.60
9 week	$62.87^{a,b,c}$	3.07	63.56^{b}	1.28	64.05^{b}	0.82	62.57^{b}	0.83	59.16^{c}	2.35	$55.20^{a,b}$	4.02
10 week	$61.58^{a,b,c}$	4.51	63.24^{b}	1.31	63.75^{b}	0.91	62.45^{b}	0.9	59.05^{c}	2.54	61.92^{b}	4.48
11 week	$61.74^{a,b,c}$	4.11	63.18^{b}	1.38	63.77^{b}	1	62.59^{b}	0.84	58.67^{c}	2.69	61.92^{b}	4.72
12 week	$61.47^{a,b,c}$	4.49	62.53^{b}	1.44	63.50^{b}	1.05	62.45^{b}	0.86	58.98^{c}	2.79	62.25^{b}	4.83
13 week	$61.76^{a,b,c}$	4.05	62.75^{b}	1.62	63.44^{b}	1.01	62.33^{b}	1.06	59.14^{c}	2.73	62.40^{b}	5.05

	pH 7	SD	pH 8	SD	pH 9	SD	pH 10	SD	pH 11	SD
0 day	24.16^{a}	0.91	11.21^{a}	0.41	5.83^{a}	0.21	4.66^{a}	0.43	5.44^{a}	1.70
1 day	30.74^{b}	2.89	12.39^{a}	0.62	7.25^{a}	0.11	7.57^{a}	1.33	10.15^{a}	2.16
1 week	56.57^{c}	0.64	41.70^{b}	1.07	43.05^{b}	2.81	44.60^{b}	0.33	46.86^{b}	3.12
2 week	57.40^{c}	0.51	50.22^{c}	2.55	53.80^{c}	2.02	$54.94^{b,c}$	0.71	55.39^{c}	2.15
3 week	$58.52^{c,d}$	0.94	$55.71^{c,d}$	0.93	$60.15^{c,d}$	2.03	$60.99^{c,d}$	0.29	$60.56^{c,d}$	2.06
4 week	$58.74^{c,d}$	1.01	$58.87^{d,e}$	0.44	$63.79^{d,e}$	2.15	$64.35^{c,d,e}$	4.31	$63.56^{d,e}$	2.02
5 week	$59.07^{c,d}$	1.07	$61.24^{d,e,f}$	0.97	$66.47^{d,e,f}$	2.35	$66.61^{d,e}$	0.04	$65.65^{d,e,f}$	2.16
6 week	$59.84^{c,d}$	1.35	$63.23^{e,f,g}$	1.31	$68.56^{e,f,g}$	2.37	$68.66^{d,e}$	3.83	$67.60^{d,e,f,g}$	2.03
7 week	$60.09^{c,d}$	1.50	$64.42^{e,f,g}$	1.59	$69.83^{e,f,g}$	2.40	$69.96^{d,e}$	3.64	$68.81^{e,f,g}$	1.95
8 week	$57.48^{c,d}$	0.88	$66.43^{f,g}$	1.93	$72.13^{f,g}$	2.59	$72.01^{d,e}$	3.32	$70.80^{e,f,g}$	1.69
9 week	$60.95^{c,d}$	1.80	$67.27^{f,g}$	2.10	$72.93^{f,g}$	2.61	72.84^{e}	3.24	$71.49^{f,g}$	1.64
10 week	$61.03^{c,d}$	1.88	$67.89^{f,g}$	2.2	$71.41^{f,g}$	3.55	73.57^{e}	3.14	$72.18^{f,g}$	1.52
11 week	$61.02^{c,d}$	1.61	68.50^{g}	2.24	74.21^{g}	2.72	74.93^{e}	3.02	$72.84^{f,g}$	1.33
12 week	$61.27^{c,d}$	1.63	68.95^{g}	2.28	74.69^{g}	2.73	74.74^{e}	2.96	73.09^{g}	1.33
13 week	63.48^{d}	2.34	63.93^{g}	2.59	75.23^{g}	2.87	75.26^{e}	2.87	73.51^{g}	0.92

Table VII Statistical analysis of chroma of sorghum TX430 Black at different pHs. ($p<0.05$)

	pH 1	SD	pH 2	SD	pH3	SD	pH 4	SD	pH 5	SD	pH 6	SD
0 day	108.02^{a}	9.13	111.26^{a}	0.24	110.59^{a}	0.37	102.32^{b,c}	0.62	90.73^{a,b}	1.35	84.15^{b}	3.19
1 day	111.81^{a}	2.60	111.72^{a}	0.33	111.41^{a,b}	0.48	103.94^{b}	0.54	94.92b	2.06	89.07^{b}	3.20
1 week	116.72^{a}	0.69	116.95e	0.58	116.67^{c}	0.80	97.59^{a,b}	0.21	76.50^{a,b}	4.35	62.93^{a}	3.13
2 week	117.68^{a}	0.47	116.81^{d,e}	0.40	115.83^{c}	0.68	96.90^{a}	0.16	76.08^{a}	4.84	62.08^{a}	3.12
3 week	117.47^{a}	0.91	116.64^{c,d,e}	1.00	116.08^{c}	0.68	96.67^{a}	0.16	75.88^{a}	5.27	61.90^{a}	3.51
4 week	117.33^{a}	1.64	116.06^{b,c,d,e}	1.17	115.20^{b,c}	1.00	96.27^{a}	0.01	75.48^{a}	5.60	61.79^{a}	3.43
5 week	116.94^{a}	1.94	115.94^{b,c,d,e}	1.18	115.35^{b,c}	1.17	96.27^{a}	0.04	75.68^{a}	5.76	62.04^{a}	3.60
6 week	116.57^{a}	2.31	115.12^{b,c,d,e}	1.37	115.59^{c}	1.18	95.82^{a}	1.69	75.17^{a}	6.26	62.10^{a}	3.64
7 week	116.36^{a}	2.10	115.84^{b,c,d,e}	0.50	155.33^{b,c}	1.46	95.65^{a}	1.86	74.89^{a}	6.50	62.26^{a}	3.93
8 wee	115.18^{a}	0.37	115.61^{b,c,d,e}	0.51	115.07^{b,c}	1.57	95.11^{a}	2.09	74.58^{a}	7.13	62.92^{a}	4.59
9 week	114.78^{a}	3.28	115.43^{b,c,d}	0.54	115.08^{b,c}	1.68	95.05^{a}	2.29	74.68^{a}	7.22	63.25^{a}	4.86
10 week	112.81^{a}	5.69	115.29^{b,c}	0.42	114.848^{b,c}	1.66	95.32^{a}	2.10	74.98^{a}	7.51	63.66^{a}	5.25
11 week	113.01^{a}	4.97	115.15^{b}	0.43	114.88^{b,c}	1.71	94.62^{a}	2.29	74.54^{a}	7.52	63.73^{a}	5.48
12 week	112.79^{a}	5.52	114.87^{b}	0.45	114.66^{b,c}	1.75	94.87^{a}	2.39	74.82^{a}	7.94	64.14^{a}	5.98
13 week	113.47^{a}	4.80	114.92^{b}	0.53	114.72^{b,c}	1.83	94.29^{a}	2.48	74.72^{a}	8.18	64.41^{a}	6.07

	pH 7	SD	pH 8	SD	pH 9	SD	pH 10	SD	pH 11	SD
0 day	78.78^{e}	1.83	70.27^{e}	0.66	62.12^{a}	2.44	57.75^{a}	3.65	55.87^{a}	8.77
1 day	79.75^{e}	1.76	69.24^{d,e}	2.14	62.79^{a}	0.82	61.65^{a}	3.03	63.70^{a}	2.43
1 week	58.69^{a,b}	2.87	60.65^{a}	1.28	62.08^{a}	0.32	60.21^{a}	5.70	61.26^{a}	1.65
2 week	58.31^{a}	2.55	61.38^{a,b}	2.37	63.40^{a}	1.18	62.20^{a}	6.10	62.34^{a}	2.31
3 week	58.78^{a,b}	2.33	63.13^{a,b,c}	2.51	64.43^{a}	1.80	63.54^{a}	6.33	63.27^{a}	1.73
4 week	59.34^{a,b,c}	2.21	64.12^{a,b,c}	2.44	64.62^{a}	2.22	63.85^{a}	6.46	60.96^{a}	1.02
5 week	59.97^{a,b,c,d,}	2.05	64.91^{a,b,c,d}	2.15	64.45^{a}	2.61	63.78^{a}	6.49	63.85^{a}	0.42
6 week	60.83^{a,b,c,d}	1.94	65.73^{b,c,d,e}	1.83	64.42^{a}	2.82	63.78^{a}	6.56	64.13^{a}	4.43
7 week	61.42^{a,b,c,d}	1.88	66.11^{b,c,d,e}	1.44	64.17^{a}	3.08	63.46^{a}	6.53	64.04^{a}	4.29
8 wee	62.71^{a,b,c,d}	1.85	66.73^{c,d,e}	0.81	63.61^{a}	3.59	62.97^{a}	6.46	63.99^{a}	4.11
9 week	63.50^{a,b,c,d}	1.61	67.05^{c,d,e}	0.47	62.69^{a}	4.43	62.57^{a}	6.47	63.87^{a}	4.05
10 week	64.15^{a,b,c,d}	1.60	67.22^{c,d,e}	0.21	65.17^{a}	4.04	62.31^{a}	6.40	63.82^{a}	4.01
11 week	64.61^{b,c,d}	1.65	67.40^{c,d,e}	0.19	62.82^{a}	4.18	62.13^{a}	6.31	63.67^{a}	4.04
12 week	65.26^{c,d}	1.55	66.10^{b,c,d,e}	2.43	62.43^{a}	4.33	61.69^{a}	6.37	63.36^{a}	4.03
13 week	65.73^{d}	1.58	67.50^{c,d,e}	0.71	62.36^{a}	4.54	61.26^{a}	6.35	63.20^{a}	3.97

Table VIII Statistical analysis of hue of sorghum TX430 Black at different pHs. ($p<0.05$)

	pH 1	SD	pH 2	SD	pH3	SD	pH 4	SD	pH 5	SD	pH 6	SD
0 day	59.27^{a}	0.06	54.90^{a}	0.20	54.70^{a}	0.46	49.03^{a}	0.38	42.13^{a}	1.58	37.27^{a}	2.87
1 day	59.37^{a}	0.55	55.17^{a}	0.31	55.13^{a}	0.57	50.70^{b}	0.30	47.80^{b}	1.15	$44.27^{a,b}$	3.14
1 week	66.40^{d}	0.44	64.43^{e}	0.12	64.67^{d}	0.42	59.40^{e}	0.10	53.67^{c}	1.00	$52.00^{b,c}$	1.39
2 week	$65.83^{c,d}$	0.42	$64.20^{d,e}$	0.10	64.53^{d}	0.40	59.27^{de}	0.15	53.43^{c}	1.01	$52.67^{b,c}$	1.60
3 week	$65.50^{b,c,d}$	0.52	$64.07^{d,e}$	0.15	$64.43^{c,d}$	0.35	$59.27^{d,e}$	0.15	53.30^{c}	0.89	$53.17^{b,c}$	2.12
4 week	$65.20^{b,c,d}$	0.53	$63.87^{c,d,e}$	0.25	$64.23^{c,d}$	0.32	$59.10^{c,d,e}$	0.10	52.93^{c}	0.97	$53.43^{b,c}$	2.49
5 week	$65.03^{b,c,d}$	0.49	$63.67^{b,c,d,e}$	0.25	$64.10^{b,c,d}$	0.26	$59.07^{c,d,e}$	0.15	52.57^{c}	1.06	$53.50^{b,c}$	3.03
6 week	$64.47^{b,c}$	0.06	$63.43^{b,c,d,e}$	0.31	$63.90^{b,c,d}$	0.36	$58.97^{c,d,e}$	0.15	52.57^{c}	0.83	$54.17^{b,c}$	3.15
7 week	$64.70^{b,c}$	0.53	$63.30^{b,c,d,e}$	0.36	$63.73^{b,c,d}$	0.35	$59.00^{c,d,e}$	0.17	52.50^{c}	0.79	$54.60^{b,c}$	3.49
8 week	$64.50^{b,c}$	0.56	$63.10^{b,c,d}$	0.46	$63.57^{b,c,d}$	0.42	$58.93^{c,d,e}$	0.12	52.37^{c}	0.64	55.33^{c}	3.93
9 week	$64.33^{b,c}$	0.61	$63.00^{b,c,d}$	0.46	$63.47^{b,c,d}$	0.42	$58.93^{c,d,e}$	0.12	52.37^{c}	0.64	55.80^{c}	4.23
10 week	64.07^{b}	0.75	$62.73^{b,c}$	0.57	$63.23^{b,c}$	0.38	$58.83^{c,d}$	0.12	52.23^{c}	0.59	56.03^{c}	4.40
11 week	64.13^{b}	0.75	$62.77^{b,c}$	0.61	$63.27^{b,c}$	0.40	$58.93^{c,d,e}$	0.12	52.47^{c}	0.47	56.53^{c}	4.50
12 week	63.90^{b}	0.75	62.53^{b}	0.67	63.00^{b}	0.44	58.73^{c}	0.12	52.33^{c}	0.45	56.80^{c}	2.57
13 week	63.87^{b}	0.75	62.47^{b}	0.71	62.90^{b}	0.44	$58.77^{c,d}$	0.15	52.47^{c}	0.40	57.17^{c}	2.48

	pH 7	SD	pH 8	SD	pH 9	SD	pH 10	SD	pH 11	SD
0 day	31.13^{a}	0.85	15.6^{a}	0.78	9.13^{a}	0.35	7.8^{a}	0.57	8.8^{a}	2.19
1 day	38.30^{b}	3.22	17.43^{a}	0.99	11.30^{a}	0.35	11.8^{a}	1.63	15.07^{a}	2.97
1 week	49.80^{c}	0.52	41.00^{b}	2.78	46.37^{b}	0.06	47.90^{b}	3.18	48.57^{b}	2.94
2 week	$51.67^{c,d}$	0.50	52.57^{c}	0.42	57.67^{c}	0.59	59.47^{c}	2.44	58.33^{c}	3.12
3 week	$53.20^{c,d,e}$	1.14	$58.73^{c,d}$	1.25	63.63^{d}	0.72	$64.80^{c,d}$	2.10	$63.50^{c,d}$	2.62
4 week	$54.00^{c,d,e,f}$	1.42	$62.37^{d,e}$	1.97	$66.97^{d,e}$	0.85	$64.37^{c,d}$	3.87	$66.43^{d,e}$	2.35
5 week	$54.70^{c,d,e,f}$	1.61	$64.90^{d,e,f}$	2.34	$69.40^{e,f}$	1.06	$69.70^{d,e}$	1.73	$68.50^{d,e,f}$	2.07
6 week	$55.63^{c,d,e,f}$	1.91	$66.57^{e,f,g}$	2.31	$70.91^{f,g}$	1.11	$71.00^{d,e,f}$	1.74	$69.93^{d,e,f}$	1.82
7 week	$56.37^{c,d,e,f}$	2.04	$67.87^{e,f,g}$	2.40	$72.07^{f,g,h}$	1.19	$72.20^{f,g}$	1.65	$71.10^{d,e,f}$	1.61
8 week	$57.7^{d,e,f}$	2.29	$69.70^{f,g}$	2.34	$73.90^{g,h,i}$	1.39	$73.73^{f,g}$	1.62	$72.83^{f,g}$	1.22
9 week	$58.27^{e,f}$	2.39	$70.57^{f,g}$	2.22	$74.67^{h,i}$	1.46	$74.53^{f,g}$	1.62	$73.50^{f,g}$	1.18
10 week	$58.77^{e,f}$	2.56	$71.10^{f,g}$	2.17	$74.10^{g,h,i}$	1.76	$75.00^{f,g}$	1.56	$73.96^{f,g}$	1.01
11 week	$59.33^{e,f}$	2.50	$71.60^{f,g}$	2.08	$75.70^{h,i}$	1.48	$75.70^{f,g}$	1.56	74.70^{g}	0.95
12 week	$59.73^{e,f}$	2.57	72.07^{g}	2.05	76.07^{i}	1.54	$75.97^{f,g}$	1.50	74.83^{g}	1.00
13 week	60.37^{f}	2.48	71.44^{g}	2.38	76.47^{i}	1.71	76.50^{f}	1.47	75.37^{g}	0.55

Table IX Statistical analysis of lightness of sorghum Black PI Tall at different pHs. (p<0.05)

	pH 1	SD	pH 2	SD	pH3	SD	pH 4	SD	pH 5	SD	pH 6	SD
0 day	40.11^{g}	1.12	40.65^{j}	0.18	36.04^{a}	0.69	34.09^{a,b,c,d}	0.20	34.23^{a,b}	0.45	34.88^{a,b}	1.20
1 day	39.47^{g}	0.91	40.63^{i,j}	0.30	36.91^{a}	0.76	35.75^{a,b,c,d}	0.21	35.82^{a,b}	0.59	36.66^{a,b}	0.54
1 week	37.24^{f,g}	0.43	39.45^{h,I,j}	0.66	37.26^{a}	0.77	36.44^{d}	0.34	36.52^{b}	0.71	37.53^{b}	1.16
2 week	35.76^{e,f}	0.43	38.58^{g,h,I,j}	0.75	36.79^{a}	0.84	35.78^{b,c,d}	0.49	35.81^{a,b}	0.91	36.87^{a,b}	1.08
3 week	34.74^{d,e,f}	0.47	37.97^{f,g,h,I,j}	0.87	36.62^{a}	1.02	35.82^{c,d}	0.73	35.75^{a,b}	0.99	37.05^{a,b}	1.04
4 week	33.83^{c,d,e,f}	0.68	37.25^{e,f,g,h}	0.80	36.08^{a}	1.06	35.23^{a,b,c,d}	0.79	35.09^{a,b}	1.13	36.43^{a,b}	1.02
5 week	32.91^{c,d,e}	0.74	36.48^{d,e,f,g}	0.68	35.64^{a}	1.10	34.86^{a,b,c,d}	0.87	34.57^{a,b}	1.25	36.63^{a,b}	1.03
6 week	32.55^{c,d,e}	0.79	36.09^{c,d,e,f,g}	0.69	35.54^{a}	1.29	34.72^{a,b,c,d}	0.92	34.37^{a,b}	1.41	35.77^{a,b}	1.00
7 week	32.25^{b,c,d,e}	0.97	35.65^{c,d,e,f}	0.65	35.28^{a}	1.37	34.34^{a,b,c,d}	1.04	33.87^{a,b}	1.55	35.60^{a,b}	0.92
8 week	31.69^{a,b,c,d}	1.09	35.12^{c,d,e}	0.67	35.10^{a}	1.50	34.22^{a,b,c,d}	1.08	33.66^{a,b}	1.62	35.52^{a,b}	0.91
9 week	31.10^{a,b,c}	1.22	34.60^{b,c,d}	0.66	34.04^{a}	1.69	33.92^{a,b,c,d}	1.17	33.19^{a,b}	1.81	35.14^{a,b}	0.81
10 week	30.60^{a,b,c}	1.33	34.01^{a,b,c,d}	0.71	34.50^{a}	1.76	33.55^{a,b,c,d}	1.22	32.78^{a,b}	1.94	34.96^{a,b}	0.83
11 week	30.40^{a,b,c}	1.45	33.76^{a,b,c}	0.77	34.39^{a}	1.84	33.49^{a,b,c}	1.25	32.62^{a,b}	2.12	34.95^{a,b}	0.78
12 week	28.29^{a}	2.39	32.35^{a,b}	1.37	33.78^{a}	2.36	32.97^{a,b}	1.46	31.48^{a}	2.47	34.51^{a}	0.70
13 week	28.67^{a,b}	2.03	31.97^{a}	1.63	33.68^{a}	2.54	32.83^{a}	1.45	31.36^{a}	2.86	34.58^{a,b}	0.66

	pH 7	SD	pH 8	SD	pH 9	SD	pH 10	SD	pH 11	SD
0 day	36.94^{a,b}	0.08	34.69^{a,b}	0.23	34.37^{a,b}	0.24	34.23^{a}	0.75	33.72^{a}	1.13
1 day	38.77^{a,b}	0.70	36.48^{b,c,d}	0.23	42.47^{a,b,C}	0.10	36.22^{a}	0.75	35.36^{a}	1.30
1 week	39.42^{b}	0.80	37.47^{d}	0.31	36.98^{c}	0.14	37.19^{a}	0.66	36.17^{a}	1.78
2 week	38.67^{a,b}	0.81	37.06^{c,d}	0.27	36.63^{b,c}	0.18	36.80^{a}	0.52	35.98^{a}	2.00
3 week	38.52^{a,b}	0.94	37.00^{c,d}	0.38	36.46^{b,c}	0.26	36.60^{a}	0.53	35.87^{a}	2.04
4 week	37.80^{a,b}	0.91	36.41^{b,c,d}	0.34	35.94^{a,b,c}	0.41	36.07^{a}	2.00	35.45^{a}	2.10
5 week	37.35^{a,b}	0.93	36.02^{a,b,c,d}	0.69	35.55^{a,b,c}	1.64	35.66^{a}	1.03	35.07^{a}	2.15
6 week	37.15^{a,b}	1.03	35.91^{a,b,c,d}	0.35	35.46^{a,b,c}	0.62	35.53^{a}	2.50	35.06^{a}	2.24
7 week	36.85^{a,b}	1.12	35.67^{a,b,c,d}	0.36	35.10^{a,b,c}	0.73	35.08^{a}	2.62	34.76^{a}	2.19
8 week	36.55^{a,b}	0.23	35.47^{a,b,c}	0.39	34.94^{a,b,c}	0.84	34.81^{a}	3.03	34.59^{a}	2.30
9 week	36.21^{a,b}	1.31	35.11^{a,b,c}	0.28	34.66^{a,b,c}	0.93	34.50^{a}	3.49	34.60^{a}	2.39
10 week	35.85^{a,b}	1.39	34.90^{a,b}	0.29	34.37^{a,b}	1.00	34.15^{a}	3.79	34.41^{a}	2.44
11 week	35.75^{a}	1.52	34.86^{a,b}	0.31	34.31^{a,b}	1.03	34.02^{a}	3.95	34.40^{a}	2.51
12 week	35.18^{a}	1.86	35.30^{a,b,c}	2.11	33.79^{a}	1.38	33.26^{a}	4.99	34.16^{a}	2.82
13 week	35.17^{a}	1.94	34.30^{a}	0.43	33.78^{a}	1.45	33.08^{a}	5.48	34.23^{a}	2.92

Table X Statistical analysis of chroma of sorghum Black PI Tall at different pHs. ($p<0.05$)

	pH 1	SD	pH 2	SD	pH3	SD	pH 4	SD	pH 5	SD	pH 6	SD
0 day	98.06^{f}	1.05	98.22^{h}	0.42	92.35^{a}	0.92	90.28^{a}	0.23	$90.38^{a,b}$	0.44	91.02^{a}	1.35
1 day	$97.49^{i,j}$	0.87	$97.93^{g,h}$	1.14	93.10^{a}	1.01	87.91^{a}	0.40	91.95^{b}	0.54	92.79^{a}	1.31
1 week	$95.14^{d,e,f}$	0.65	96.55f,g,h	1.66	93.31^{a}	1.02	91.88^{a}	0.79	92.43^{b}	0.65	93.44^{a}	0.98
2 week	$90.09^{a,b,c,d}$	5.28	$95.61^{e,f,g,h}$	1.69	92.64^{a}	1.10	92.30^{a}	0.98	$91.43^{a,b}$	0.88	92.38^{a}	0.94
3 week	$92.43^{c,d,e}$	0.70	$94.94^{d,e,f,g,h}$	1.74	92.65^{a}	1.26	91.24^{a}	1.32	$91.80^{a,b}$	0.89	93.12^{a}	0.94
4 week	$91.25^{b,c,d}$	0.94	$94.24^{b,c,d,e}$	1.62	91.95^{a}	1.38	91.68^{a}	1.41	$90.88^{a,b}$	1.08	92.27^{a}	0.83
5 week	$89.81^{a,b,c}$	0.90	$93.16^{b,c,d,e,f}$	1.37	91.24^{a}	1.40	90.87^{a}	1.50	$90.18^{a,b}$	1.25	91.70^{a}	0.90
6 week	$89.50^{a,b,c}$	0.92	$92.80^{b,c,d,e,f}$	1.39	91.19^{a}	1.65	90.30^{a}	1.53	$90.02^{a,b}$	1.38	91.68^{a}	0.83
7 week	$89.18^{a,b,c}$	1.07	$92.36^{a,b,c,d,e}$	1.35	90.88^{a}	1.77	90.16^{a}	1.71	$89.30^{a,b}$	1.66	91.14^{a}	0.68
8 wee	$88.45^{a,b,c}$	1.21	$91.79^{a,b,c,d,e}$	1.25	90.69^{a}	1.91	89.65^{a}	1.73	$88.19^{a,b}$	1.66	91.24^{a}	0.74
9 week	$87.72^{a,b,c}$	1.30	$91.28^{a,b,c,d}$	1.05	90.41^{a}	2.14	89.60^{a}	1.85	$88.61^{a,b}$	1.86	90.83^{a}	0.36
10 week	$87.06^{a,b,c}$	1.50	$90.51^{a,b,c}$	0.96	89.91^{a}	2.22	89.20^{a}	1.71	$87.07^{a,b}$	2.02	90.44^{a}	0.65
11 week	$86.76^{a,b}$	1.59	$90.25^{a,b}$	0.87	89.78^{a}	2.36	88.80^{a}	1.93	$87.88^{a,b}$	2.32	90.45^{a}	0.60
12 week	84.87^{a}	2.11	88.87^{a}	0.94	89.05^{a}	2.93	88.12^{a}	2.22	86.38^{a}	2.62	89.93^{a}	0.51
13 week	84.57^{a}	2.15	88.44^{a}	1.39	89.01^{a}	3.18	87.80^{a}	2.16	86.43^{a}	3.20	90.14^{a}	0.47

	pH 7	SD	pH 8	SD	pH 9	SD	pH 10	SD	pH 11	SD
0 day	$93.09^{a,b,c,d}$	0.55	$91.05^{b,c,d}$	0.28	$90.41^{a,b,c,d,e}$	0.35	90.34^{a}	1.44	89.38^{a}	2.00
1 day	$94.78^{c,d}$	0.64	$92.87^{f,g}$	0.22	$92.07^{c,d,e}$	0.19	92.33^{a}	1.12	90.92^{a}	1.44
1 week	95.08^{d}	0.70	93.72^{g}	0.32	92.77^{e}	0.14	93.05^{a}	1.39	91.48^{a}	1.67
2 week	$94.04^{b,c,d}$	0.60	$93.14^{f,g}$	0.32	$92.31^{d,e}$	0.17	92.53^{a}	1.69	91.34^{a}	1.82
3 week	$94.17^{b,c,d}$	0.80	$93.34^{f,g}$	0.36	$92.29^{d,e}$	0.27	92.48^{a}	2.06	91.39^{a}	2.17
4 week	$93.22^{a,b,c,d}$	0.77	$91.53^{e,f}$	0.31	$91.65^{b,c,d,e}$	0.45	91.82^{a}	2.39	90.83^{a}	2.16
5 week	$92.66^{a,b,c,d}$	0.84	$91.94^{d,e}$	0.83	$91.40^{a,b,c,d,e}$	1.96	91.25^{a}	1.27	90.31^{a}	2.55
6 week	$92.51^{a,b,c,d}$	0.90	$91.87^{c,d,e}$	0.29	$90.77^{a,b,c,d,e}$	0.61	91.11^{a}	3.04	90.27^{a}	2.68
7 week	$92.04^{a,b,c,d}$	1.05	$91.55^{c,d}$	0.28	$90.69^{a,b,c,d,e}$	0.97	90.47^{a}	3.13	89.89^{a}	2.56
8 wee	$91.77^{a,b,c,d}$	1.19	$91.43^{b,c,d}$	0.41	$90.49^{a,b,c,d,e}$	0.81	90.23^{a}	3.64	89.74^{a}	2.72
9 week	$91.41^{a,b,c}$	1.22	$90.99^{b,c}$	0.18	$90.09^{a,b,c,d}$	0.95	89.82^{a}	4.30	89.70^{a}	2.79
10 week	$90.85^{a,b}$	1.36	$90.63^{a,b}$	0.24	$89.71^{a,b,c}$	1.05	89.36^{a}	4.64	89.42^{a}	2.87
11 week	$90.77^{a,b}$	1.54	$90.57^{a,b}$	0.23	$89.66^{a,b,c}$	1.09	89.21^{a}	4.89	89.40^{a}	2.95
12 week	90.21^{a}	1.83	89.94^{a}	0.32	89.10^{a}	1.46	88.33^{a}	6.03	89.17^{a}	3.31
13 week	90.33^{a}	1.94	90.08^{a}	0.38	$89.30^{a,b}$	1.54	88.23^{a}	6.69	89.34^{a}	3.38

Table XI Statistical analysis of hue of sorghum Black PI Tall at different pHs. ($p<0.05$)

	pH 1	SD	pH 2	SD	pH3	SD	pH 4	SD	pH 5	SD	pH 6	SD
0 day	48.87^{h}	1.04	45.60^{i}	0.56	42.27^{a}	0.46	40.43^{a,b}	0.36	40.70^{a}	0.44	41.27^{a}	1.12
1 day	44.20^{g,h}	0.87	45.47^{i}	0.32	43.07^{a}	0.55	42.10^{b,c,d,e}	0.17	42.17^{a}	0.55	42.87^{a}	1.19
1 week	42.40^{f,g,h}	0.30	44.73^{h,i}	0.06	43.47^{a}	0.57	42.83^{e}	0.06	42.80^{a}	0.70	43.77^{a}	1.19
2 week	41.27^{e,f,g}	0.25	43.53^{f,g,h,i}	0.10	43.13^{a}	0.61	42.47^{d,e}	0.21	42.40^{a}	0.85	43.40^{a}	1.01
3 week	40.33^{e,f,g}	0.31	42.90^{e,f,g,h}	0.23	42.90^{a}	0.75	42.30^{c,d,e}	0.36	42.10^{a}	0.90	43.23^{a}	0.95
4 week	39.63^{c,d,e,f}	0.45	42.40^{d,e,f,g}	0.26	42.57^{a}	0.75	41.93^{b,c,d,e}	0.35	41.67^{a}	1.05	42.83^{a}	1.01
5 week	39.13^{c,d,e}	0.59	42.07^{c,d,e,f,g}	0.20	42.27^{a}	0.81	41.63^{a,b,c,d,e}	0.45	41.30^{a}	1.15	42.57^{a}	0.96
6 week	38.77^{b,c,d,e}	0.64	41.73^{c,d,e,f}	0.32	42.17^{a}	0.97	41.57^{a,b,c,d,e}	0.50	41.10^{a}	1.30	42.47^{a}	0.90
7 week	38.50^{a,b,c,d,e}	0.87	41.23^{c,d,e}	0.38	41.93^{a}	1.03	41.27^{a,b,c,d,e}	0.60	40.77^{a}	1.35	42.30^{a}	0.95
8 week	38.07^{a,b,c,d}	0.99	40.73^{b,c,d}	0.50	41.80^{a}	1.15	41.13^{a,b,c,d,e}	0.61	40.53^{a}	1.50	42.10^{a}	0.92
9 week	37.63^{a,b,c,d}	1.12	40.33^{a,b,c,d}	0.65	41.53^{a}	1.27	40.90^{a,b,c,d}	0.72	40.17^{a}	1.65	41.90^{a}	0.82
10 week	37.03^{a,b,c}	0.87	40.10^{a,b,c}	0.71	41.33^{a}	1.34	40.67^{a,b,c}	0.76	39.87^{a}	1.75	41.77^{a}	0.83
11 week	37.10^{a,b,c}	1.25	38.87^{a,b}	0.82	41.27^{a}	1.38	40.60^{a,b,c}	0.82	39.73^{a}	1.90	41.73^{a}	0.78
12 week	35.93^{a,b}	1.76	38.47^{a}	1.44	40.80^{a}	1.84	40.13^{a}	0.97	38.83^{a}	2.30	41.40^{a}	0.78
13 week	35.67^{a}	1.86	38.47^{a}	1.70	40.67^{a}	1.97	40.00^{a}	0.98	38.63^{a}	2.50	41.37^{a}	0.72

	pH 7	SD	pH 8	SD	pH 9	SD	pH 10	SD	pH 11	SD
0 day	43.00^{a,b}	0.07	41.03^{a,b}	0.21	40.87^{a,b}	0.21	40.73^{a}	0.71	40.53^{a}	1.21
1 day	44.83^{a,b}	0.76	42.57^{d,e,f,g}	0.14	42.47^{a,b,c,d}	0.21	42.50^{a}	0.64	42.07^{a}	1.06
1 week	45.70^{b}	0.78	43.50^{g}	0.20	43.33^{d}	0.21	43.47^{a}	0.95	42.90^{a}	1.35
2 week	45.17^{a,b}	0.90	43.27^{f,g}	0.23	43.10^{c,d}	0.17	43.23^{a}	1.06	42.70^{a}	1.55
3 week	44.83^{a,b}	1.02	43.00^{e,f,g}	0.35	42.87^{b,c,d}	0.25	42.97^{a}	1.23	42.50^{a}	1.65
4 week	44.30^{a,b}	0.96	42.67^{d,e,f,g}	0.32	42.50^{a,b,c,d}	0.36	42.57^{a}	1.57	42.23^{a}	1.66
5 week	44.13^{a,b}	0.84	42.47^{c,d,e,f}	0.55	42.33^{a,b,c,d}	1.34	42.27^{a}	0.75	41.97^{a}	1.70
6 week	43.77^{a,b}	1.00	42.33^{c,d,e,f}	0.31	42.00^{a,b,c,d}	0.50	42.13^{a}	1.95	41.97^{a}	1.76
7 week	43.57^{a,b}	1.00	42.17^{c,d,e}	0.32	41.90^{a,b,c,d}	0.66	41.87^{a}	2.06	41.73^{a}	1.76
8 week	43.33^{a,b}	1.12	41.97^{b,c,d}	0.35	41.70^{a,b,c,d}	0.75	41.63^{a}	2.38	41.57^{a}	1.86
9 week	43.03^{a,b}	1.21	41.77^{a,b,c,d}	0.35	41.47^{a,b,c,d}	0.85	41.37^{a}	2.75	41.60^{a}	1.97
10 week	42.80^{a,b}	1.23	41.57^{a,b,c}	0.35	41.30^{a,b,c,d}	0.90	41.07^{a}	3.09	41.50^{a}	1.97
11 week	42.70^{a,b}	1.32	41.53^{a,b,c}	0.35	41.23^{a,b,c,d}	0.90	41.00^{a}	3.12	41.47^{a}	2.02
12 week	42.20^{a}	1.68	41.07^{a,b}	0.40	40.77^{a}	1.20	40.30^{a}	4.07	41.30^{a}	2.29
13 week	42.13^{a}	1.74	41.00^{a}	0.46	40.67^{a}	1.30	40.07^{a}	4.47	41.27^{a}	2.35

Table XII Statistical analysis of lightness of sorghum Tx430 Black at different temperatures. ($p<0.05$)

	-8 C	SD	4 C	SD	25 C	SD	50 C	SD
0 day	52.30[a]	0.42	51.74[a]	0.62	51.78[a]	1.71	52.89[a]	0.46
1 day	52.50[a]	0.37	51.95[a]	0.66	52.16[a]	1.65	52.11[a]	0.74
1 week	52.69[a]	0.46	52.07[a]	0.62	52.09[a]	1.57	47.02[a]	2.27
2 week	65.22[b]	0.45	64.64[b]	0.51	64.71[b]	1.35	59.06[a]	3.83
3 week	64.14[b]	0.56	64.55[b]	0.49	63.94[b]	1.24	57.63[a]	1.80
4 week	65.15[b]	0.54	64.48[b]	0.57	63.75[b]	1.34	55.25[a]	2.91
5 week	65.06[b]	0.47	64.36[b]	0.56	63.54[b]	1.10	65.52[a]	4.56
6 week	65.20[b]	0.53	64.53[b]	0.53	63.56[b]	0.91	59.49[a]	4.10
7 week	64.98[b]	0.64	64.24[b]	0.53	63.13[b]	0.76	60.14[a]	5.77
8 week	65.51[b]	0.65	64.66[b]	0.64	63.37[b]	0.58	60.34[a]	5.88
9 week	65.14[b]	0.65	64.13[b]	0.66	62.51[b]	0.25	58.86[a]	7.79
10 week	65.32[b]	0.62	64.22[b]	0.76	62.38[b]	0.16	58.71[a]	8.80
11 week	65.10[b]	0.82	64.06[b]	0.78	61.93[b]	0.06	58.34[a]	8.04
12 week	65.04[b]	0.91	63.92[b]	0.79	61.63[b]	0.28	57.59[a]	10.51
13 week	64.77[c]	0.97	63.53[b]	0.93	60.91[b]	0.59	57.47[a]	11.78

Table XIII Statistical analysis of chroma of sorghum Tx430 Black at different temperatures. ($p<0.05$)

	-8 C	SD	4 C	SD	25 C	SD	50 C	SD
0 day	110.53[a]	0.53	109.89[a]	0.74	109.93[a]	1.97	110.95[a]	0.43
1 day	111.09[a]	0.39	110.40[a]	0.74	110.58[a,b,c]	1.72	110.60[a]	0.83
1 week	111.05[a]	0.62	110.36[a]	0.65	110.36[a,b]	1.65	105.69[a]	2.32
2 week	117.77b	0.67	118.38[c]	0.60	115.41[d]	0.63	113.68[a]	2.47
3 week	117.37[b]	0.78	118.09[c]	0.60	115.63[d]	0.15	111.32[a]	4.11
4 week	117.10[b]	0.97	117.84[b,c]	0.61	115.20[d]	0.04	106.92[a]	8.34
5 week	116.82[b]	0.88	117.44[b,c]	0.55	115.03[d]	0.14	99.96[a]	4.61
6 week	116.73[b]	1.07	117.57[b,c]	0.62	115.38[d]	0.36	102.80[a]	5.85
7 week	116.37[b]	0.91	117.05[b,c]	0.64	115.11[d]	0.44	106.53[a]	6.27
8 week	116.83[b]	1.18	117.48[b,c]	0.81	115.59[d]	0.69	106.85[a]	6.31
9 week	115.98[b]	1.26	116.63[b,c]	0.77	114.97[d]	0.76	103.37[a]	6.27
10 week	116.13[b]	1.50	116.84[b,c]	0.65	115.19[d]	0.16	102.05[a]	8.80
11 week	115.72[b]	1.38	116.53[b,c]	0.90	114.77[c,d]	1.15	100.10[a]	7.08
12 week	115.59[b]	1.23	116.48[b,c]	0.86	114.58[b,c,d]	1.07	99.09[a]	6.11
13 week	115.27[b]	1.38	115.93[c]	0.75	113.72[a,b,c,d]	0.99	97.36[a]	6.63

Table XIV Statistical analysis of hue of sorghum Tx430 Black at different temperatures. (p<0.05)

	-8 C	SD	4 C	SD	25 C	SD	50 C	SD
0 day	54.67[a]	0.29	54.33[a]	0.42	54.20[a]	1.08	55.30[a]	0.44
1 day	54.63[a]	0.23	54.30[a]	0.46	54.47[a]	1.29	54.37[a]	0.50
1 week	54.90[a]	0.26	54.50[a]	0.46	54.50[a]	1.14	50.17[a]	1.89
2 week	64.63[b]	0.15	64.37[b]	0.38	63.93[b]	0.74	60.63[a]	2.93
3 week	64.60[b]	0.26	64.33[b]	0.32	63.40[b]	0.71	59.80[a]	1.84
4 week	64.60[b]	0.26	64.33[b]	0.32	63.35[b]	0.78	58.60[a]	2.55
5 week	64.57[b]	0.21	64.33[b]	0.32	63.20[b]	0.71	63.83[a]	3.29
6 week	64.70[b]	0.30	64.37[b]	0.31	63.10[b]	0.57	63.27[a]	3.35
7 week	64.63[b]	0.25	64.27[b]	0.31	62.85[b]	0.50	62.20[a]	4.10
8 week	64.73[b]	0.25	64.40[b]	0.30	62.85[b]	0.35	62.25[a]	4.17
9 week	64.87[b]	0.40	64.13[b]	0.31	62.40[b]	0.28	61.80[a]	5.52
10 week	64.63[b]	0.25	64.17[b]	0.32	62.25[b]	0.16	61.85[a]	8.80
11 week	64.60[b]	0.36	64.10[b]	0.35	62.00[b]	0.00	61.70[a]	5.43
12 week	64.60[b]	0.36	64.03[b]	0.38	61.85[b]	0.07	61.50[a]	7.21
13 week	64.43[b]	0.42	63.90[b]	0.35	61.60[b]	0.14	61.40[a]	7.92

Table XV Statistical analysis of lightness of sorghum Black PI Tall at different temperatures. (p<0.05)

	-8 C	SD	4 C	SD	25 C	SD	50 C	SD
0 day	40.99[b]	0.57	41.92[c]	0.47	42.64[h]	0.48	44.83[f]	2.40
1 day	40.88[b]	0.67	41.51[c]	0.78	40.9393[g,h]	0.50	39.83[e,f]	0.17
1 week	40.52[a,b]	0.76	41.13[b,c]	1.00	39.33[f,g,h]	0.53	33.26[d,e]	1.64
2 week	40.43[a,b]	0.74	40.40[a,b,c]	1.05	39.08[e,f,g]	0.57	30.81[c,d]	1.75
3 week	40.34[a,b]	0.88	40.27[a,b,c]	1.29	38.63[d,e,f,g]	0.53	29.59[b,c,d]	2.06
4 week	40.10[a,b]	0.84	39.94[a,b,c]	1.37	37.08[c,d,e,f]	0.70	27.80[a,b,c,d]	1.85
5 week	39.89[a,b]	0.89	39.80[a,b,c]	1.35	37.32[b,c,d,e,f]	0.92	26.53[a,b,c,d]	1.97
6 week	39.69[a,b]	0.95	39.62[a,b,c]	1.48	36.89[a,b,c,d,e]	1.09	25.95[a,b,c]	2.14
7 week	39.62[a,b]	0.87	39.25[a,b,c]	1.48	36.50[a,b,c,d,e]	1.22	25.33[a,b,c]	2.26
8 week	39.36[a,b]	0.89	39.00[a,b,c]	1.50	36.25[a,b,c,d,e]	1.21	24.18[a,b,c]	2.61
9 week	39.29[a,b]	1.01	38.47[a,b,c]	1.43	35.87[a,b,c,d]	1.26	23.07[a,b]	3.08
10 week	39.18[a,b]	0.94	38.16[a,b,c]	1.57	35.41[a,b,c]	1.14	22.23[a]	3.35
11 week	38.88[a,b]	0.90	37.82[a,b,c]	1.61	35.29[a,b,c]	1.22	21.96[a]	3.44
12 week	38.13[a,b]	1.07	36.78[a]	1.94	34.28[a]	1.40	26.91[a,b,c,d]	1.72
13 week	38.54[a,b]	1.05	37.18[a,b]	1.86	34.39[a,b]	1.37	26.51[a,b,c,d]	1.59

Table XVI Statistical analysis of chroma of sorghum Black PI Tall at different temperatures. (p<0.05)

	-8 C	SD	4 C	SD	25 C	SD	50 C	SD
0 day	98.83^{c}	0.57	99.83^{b}	0.83	100.53^{f}	0.43	102.96^{f}	2.39
1 day	98.76^{c}	0.64	99.35^{b}	1.20	$98.73^{e,f}$	0.66	97.90^{f}	0.14
1 week	$97.96^{b,c}$	0.83	$98.57^{a,b}$	1.61	$97.37^{d,e,f}$	0.72	89.86^{e}	1.73
2 week	$97.70^{b,c}$	0.75	$97.22^{a,b}$	1.65	$96.22^{c,d,e}$	0.72	$85.96^{d,e}$	1.90
3 week	$97.66^{b,c}$	1.05	$97.30^{a,b}$	1.95	$96.04^{c,d,e}$	0.62	$84.01^{c,d,e}$	2.35
4 week	$97.28^{a,b,c}$	0.89	$96.84^{a,b}$	2.02	$94.95^{b,c,d}$	0.80	$81.23^{b,c,d}$	2.15
5 week	$97.01^{a,b,c}$	0.98	$96.67^{a,b}$	1.96	$94.40^{a,b,c,d}$	1.12	$79.33^{a,b,c,d}$	2.39
6 week	$96.65^{a,b,c}$	1.07	$96.52^{a,b}$	2.11	$93.88^{a,b,c,d}$	1.25	$78.40^{a,b,c,d}$	2.57
7 week	$96.61^{a,b,c}$	0.92	$96.01^{a,b}$	2.09	$93.37^{a,b,c}$	1.40	$77.58^{a,b,c}$	2.53
8 week	$96.16^{a,b,c}$	0.89	$95.81^{a,b}$	2.13	$93.19^{a,b,c}$	1.34	$75.75^{a,b}$	3.15
9 week	$96.17^{a,b,c}$	1.15	$95.08^{a,b}$	1.87	$92.79^{a,b,c}$	1.35	$74.15^{a,b}$	3.67
10 week	$96.07^{a,b,c}$	0.95	$94.79^{a,b}$	2.10	$92.31^{a,b}$	1.49	73.05^{a}	4.01
11 week	$95.71^{a,b}$	0.92	$94.33^{a,b}$	2.12	$92.21^{a,b}$	1.54	72.56^{a}	4.05
12 week	94.54^{a}	1.04	93.24^{a}	2.46	91.15^{a}	1.92	$77.19^{a,b,c}$	2.20
13 week	$95.35^{a,b}$	1.02	$94.04^{a,b}$	2.29	$91.49^{a,b}$	1.85	$76.76^{a,b,c}$	1.99

Table XVII Statistical analysis of hue of sorghum Black PI Tall at different temperatures. (p<0.05)

	-8 C	SD	4 C	SD	25 C	SD	50 C	SD
0 day	45.73^{a}	0.47	46.47^{b}	0.21	47.07^{g}	0.42	48.70^{e}	1.93
1 day	45.53^{a}	0.64	46.17^{b}	0.45	$45.67^{f,g}$	0.31	$44.50^{d,e}$	0.20
1 week	45.53^{a}	0.72	46.07^{b}	0.50	$45.00^{e,f,g}$	0.44	$39.60^{c,d}$	1.44
2 week	45.53^{a}	0.74	45.80^{b}	0.60	$44.40^{d,e,f}$	0.44	$38.07^{b,c,d}$	1.58
3 week	45.43^{a}	0.72	$45.57^{a,b}$	0.76	$43.83^{c,d,e,f}$	0.40	$37.33^{a,b,c}$	1.80
4 week	45.30^{a}	0.79	$45.37^{a,b}$	0.86	$43.27^{b,c,d,e}$	0.55	$36.07^{a,b,c}$	1.60
5 week	45.20^{a}	0.79	$45.27^{a,b}$	0.86	$42.90^{b,c,d,e}$	0.70	$35.17^{a,b,c}$	1.72
6 week	45.07^{a}	0.85	$45.03^{a,b}$	0.97	$42.57^{a,b,c,d}$	0.83	$34.70^{a,b,c}$	1.95
7 week	45.00^{a}	0.79	$44.80^{a,b}$	0.98	$42.33^{a,b,c,d}$	0.97	$34.27^{a,b,c}$	2.16
8 week	44.93^{a}	0.91	$44.57^{a,b}$	1.00	$42.10^{a,b,c,d}$	0.98	$32.30^{a,b,c}$	2.60
9 week	44.77^{a}	0.85	$44.20^{a,b}$	1.08	$41.73^{a,b,c}$	1.04	$32.30^{a,b}$	3.54
10 week	44.66^{a}	0.87	$43.90^{a,b}$	1.15	$41.37^{a,b}$	0.83	31.50^{a}	3.44
11 week	44.43^{a}	0.83	$43.67^{a,b}$	1.12	$41.27^{a,b}$	0.93	31.37^{a}	3.61
12 week	43.97^{a}	0.97	$42.80^{a,b}$	1.37	40.37^{a}	1.00	$36.86^{a,b,c}$	1.50
13 week	44.10^{a}	0.98	$42.93^{a,b}$	1.37	40.37^{a}	0.99	$36.43^{a,b,c}$	1.45

Table XVIII Statistical analysis of lightness of sorghum Tx430 Black at different water activities. ($p<0.05$)

	Aw 1.0	SD	Aw 0.95	SD	Aw 0.85	SD	Aw 0.20	SD
0 day	67.98^{a}	4.64	71.94^{a}	1.54	76.20^{a}	1.34	76.5^{a}	0.36
1 day	$73.22^{a,b}$	6.38	$77.51^{b,c,d}$	2.04	80.84^{b}	2.15	82.64^{c}	0.92
1 week	$73.73^{a,b}$	3.63	$76.93^{a,b,c,d}$	2.15	80.87^{b}	1.66	$83.17^{c,d}$	0.94
2 week	$74.22^{a,b}$	7.72	$76.25^{a,b,c,d}$	2.12	80.99^{b}	0.90	$83.87^{c,d,e}$	0.52
3 week	$74.48^{a,b}$	7.46	$76.97^{a,b,c,d}$	2.12	81.30^{b}	0.92	$84.43^{d,e}$	0.41
4 week	$72.66^{a,b}$	6.50	$73.48^{a,b}$	2.41	76.14^{a}	1.68	80.51^{b}	0.37
5 week	$74.88^{a,b}$	7.44	$75.13^{a,b,c}$	2.19	80.37^{b}	0.52	$85.23^{e,f}$	0.62
6 week	$75.03^{a,b}$	6.63	$76.83^{a,b,c,d}$	2.68	81.00^{b}	0.96	$86.40^{f,g}$	0.43
7 week	$76.82^{a,b}$	7.94	$76.79^{a,b,c,d}$	2.42	81.76^{b}	0.34	$86.99^{g,h}$	0.37
8 week	$81.54^{a,b}$	8.03	$79.80^{c,d}$	1.06	81.57^{b}	0.71	$88.11^{h,i}$	0.34
9 week	$78.74^{a,b}$	7.30	$79.80^{c,d}$	0.69	83.38^{b}	1.33	$88.52^{h,i}$	0.69
10 week	$80.18^{a,b}$	1.92	$80.50^{c,d}$	1.09	82.40^{b}	0.44	$89.26^{i,j}$	0.29
11 week	$78.02^{a,b}$	3.70	$79.36^{c,d}$	1.44	81.82^{b}	0.68	$89.60^{i,j}$	0.45
12 week	$86.58^{a,b}$	4.90	81.56^{d}	0.69	81.78^{b}	2.36	90.21^{j}	0.38
13 week	87.98^{b}	4.94	$79.19^{c,d}$	0.58	83.33^{b}	1.39	90.39^{j}	0.06

Table XIX Statistical analysis of chroma of sorghum Tx430 Black at different water activities. ($p<0.05$)

	Aw 1.0	SD	Aw 0.95	SD	Aw 0.85	SD	Aw 0.20	SD
0 day	41.91^{d}	2.57	$56.83^{e,f}$	3.18	$61.31^{c,d}$	0.59	55.91^{j}	3.69
1 day	43.20^{d}	2.11	58.09^{f}	1.48	63.94^{d}	0.75	58.47^{j}	2.94
1 week	$40.96^{c,d}$	2.08	$56.13^{e,f}$	0.97	$62.81^{c,d}$	0.61	$54.27^{i,j}$	2.17
2 week	$39.06^{b,c,d}$	1.90	$54.63^{d,e,f}$	0.63	$60.95^{c,d}$	0.70	$50.29^{h,i}$	2.30
3 week	$39.04^{b,c,d}$	1.93	$53.03^{c,d,e}$	0.47	$58.88^{b,c,d}$	0.20	$47.89^{g,h}$	1.66
4 week	$34.76^{a,b,c,d}$	2.55	$50.44^{c,d}$	0.24	$57.08^{a,b,c,d}$	0.75	$45.52^{f,g,h}$	1.88
5 week	$34.29^{a,b,c,d}$	2.64	$51.28^{c,d}$	0.67	$56.80^{a,b,c,d}$	0.29	$43.35^{e,f,g}$	1.12
6 week	$33.55^{a,b,c,d}$	3.12	49.94^{c}	0.52	$56.05^{a,b,c,d}$	2.76	$41.00^{e,f}$	1.32
7 week	$32.50^{a,b,c,d}$	2.19	$49.56^{b,c}$	0.31	$57.63^{a,b,c,d}$	7.23	$38.57^{d,e}$	1.07
8 week	$29.31^{a,b,c}$	0.45	$45.66^{a,b}$	1.75	$51.97^{a,b,c}$	2.63	$34.20^{c,d}$	0.95
9 week	$32.05^{a,b,c,d}$	2.86	45.24^{a}	1.83	$49.29^{a,b}$	4.16	$32.61^{b,c}$	0.89
10 week	$27.38^{a,b}$	1.88	44.08^{a}	0.94	$49.77^{a,b}$	2.20	$31.17^{a,b,c}$	0.96
11 week	$27.61^{a,b}$	1.94	44.00^{a}	1.87	$48.97^{a,b}$	2.21	$29.50^{a,b,c}$	0.91
12 week	24.55^{a}	3.05	42.14^{a}	0.98	$48.41^{a,b}$	2.12	$28.42^{a,b}$	0.94
13 week	23.60^{a}	2.61	42.34^{a}	1.85	46.87^{a}	2.03	27.03^{a}	0.57

Table XX Statistical analysis of hue of sorghum Tx430 Black at different water activities. (p<0.05)

	Aw 1.0	SD	Aw 0.95	SD	Aw 0.85	SD	Aw 0.20	SD
0 day	72.73^{a}	2.72	$71.30^{e,f}$	0.26	$71.33^{g,h}$	0.59	$67.47^{c,d,e}$	0.38
1 day	73.07^{a}	3.19	71.97^{f}	0.15	72.23^{h}	0.75	$67.93^{d,e}$	0.25
1 week	73.47^{a}	4.03	$71.37^{e,f}$	0.31	$71.43^{g,h}$	0.61	$67.40^{b,c,d,e}$	0.20
2 week	71.77^{a}	3.82	$70.00^{d,e}$	0.36	$70.30^{f,g}$	0.70	$66.50^{b,c,d,e}$	0.26
3 week	70.67^{a}	0.75	$70.10^{d,e,f}$	0.20	$70.20^{f,g}$	0.20	$66.33^{b,c,d}$	0.32
4 week	69.73^{a}	2.90	$67.87^{b,c}$	0.50	67.80^{d}	0.75	64.37^{a}	0.29
5 week	70.77^{a}	3.72	$67.67^{b,c}$	0.35	$68.67^{d,e,f}$	0.29	$65.73^{a,b,c}$	0.32
6 week	69.50^{a}	3.29	$68.00^{b,c}$	0.52	$68.70^{d,e,f}$	0.44	$65.67^{a,b}$	0.06
7 week	72.27^{a}	3.61	$68.90^{c,d}$	0.62	$69.80^{e,f,g}$	0.00	$66.27^{b,c,d}$	0.21
8 week	72.13^{a}	3.32	$68.20^{b,c,d}$	1.00	$67.50^{c,d}$	0.20	$66.07^{a,b,c}$	0.49
9 week	67.60^{a}	0.87	$68.03^{b,c}$	1.21	$68.30^{d,e}$	0.66	$66.07^{a,b,c}$	0.64
10 week	69.77^{a}	1.80	$67.63^{b,c}$	0.31	$67.20^{b,c,d}$	0.00	$66.80^{b,c,d,e}$	0.53
11 week	68.43^{a}	2.70	$66.13^{a,b}$	1.21	$66.13^{a,b,c}$	0.21	$67.10^{b,c,d,e}$	0.70
12 week	71.20^{a}	2.08	$66.77^{a,b}$	1.15	$65.77^{a,b}$	1.10	$67.07^{b,c,d,e}$	0.76
13 week	71.50^{a}	2.01	65.67^{a}	0.74	65.30^{a}	0.46	68.17^{e}	1.58

Table XXI Statistical analysis of lightness of sorghum Black PI Tall at different water activities. (p<0.05)

	Aw 1.0	SD	Aw 0.95	SD	Aw 0.85	SD	Aw 0.20	SD
0 day	39.99^{c}	2.49	51.01^{a}	2.65	53.59^{a}	0.72	55.41^{a}	2.16
1 day	$38.20^{b,c}$	2.67	$49.38^{b,c}$	2.66	51.50^{a}	0.89	55.84^{a}	1.00
1 week	$38.43^{b,c}$	1.85	$48.95^{b,c}$	3.34	51.61^{a}	0.52	58.23^{a}	1.10
2 week	$37.44^{b,c}$	2.34	$45.95^{a,b,c}$	3.32	51.80^{a}	0.73	58.77^{a}	1.47
3 week	$36.64^{a,b,c}$	2.24	$43.57^{a,b,c}$	3.59	49.67^{a}	2.18	57.91^{a}	1.53
4 week	$36.93^{a,b,c}$	1.48	$42.67^{a,b,c}$	1.92	48.54^{a}	5.14	58.07^{a}	1.10
5 week	$36.49^{a,b,c}$	1.75	$46.40^{a,b,c}$	3.70	53.77^{a}	1.39	58.18^{a}	1.16
6 week	$36.54^{a,b,c}$	1.79	$44.75^{a,b,c}$	2.49	50.32^{a}	3.03	58.19^{a}	1.43
7 week	$36.65^{a,b,c}$	1.38	$42.54^{a,b,c}$	1.52	51.64^{a}	4.09	54.71^{a}	1.82
8 week	$34.95^{a,b,c}$	1.89	37.44^{a}	3.00	48.29^{a}	1.17	55.66^{a}	1.43
9 week	$34.59^{a,b,c}$	1.82	37.26^{a}	1.27	46.23^{a}	3.27	55.70^{a}	1.45
10 week	$33.51^{a,b}$	1.46	$40.84^{a,b}$	2.84	50.47^{a}	3.48	55.91^{a}	1.46
11 week	31.45^{a}	1.16	$40.91^{a,b}$	4.08	50.66^{a}	2.77	55.33^{a}	1.73
12 week	$33.40^{a,b}$	1.34	$42.23^{a,b,c}$	4.61	48.52^{a}	2.37	59.06^{a}	2.06
13 week	$34.47^{a,b,c}$	1.91	$42.66^{a,b,c}$	4.04	51.73^{a}	3.54	58.85^{a}	2.01

Table XXII Statistical analysis of chroma of sorghum Black PI Tall at different water activities. (p<0.05)

	Aw 1.0	SD	Aw 0.95	SD	Aw 0.85	SD	Aw 0.20	SD
0 day	82.21^{b}	3.17	97.05^{d}	3.95	104.84^{d}	0.43	$108.38^{b,c,d,e}$	1.50
1 day	$80.18^{a,b}$	3.56	$94.63^{c,d}$	3.97	$101.77^{c,d}$	0.13	$108.45^{b,c,d,e}$	0.78
1 week	80.92^{b}	2.77	$93.81^{b,c,d}$	4.82	$100.67^{b,c,d}$	0.63	112.24^{e}	0.71
2 week	$79.44^{a,b}$	3.21	$89.45^{a,b,c,d}$	4.78	$99.89^{b,c,d}$	1.51	112.01^{e}	1.17
3 week	$78.74^{a,b}$	3.04	$86.55^{a,b,c,d}$	5.21	$96.47^{a,b,c,d}$	3.42	$110.58^{d,e}$	1.35
4 week	$78.89^{a,b}$	2.02	$85.31^{a,b,c,d}$	3.14	$93.95^{a,b,c}$	6.11	$110.11^{c,d,e}$	1.15
5 week	$78.44^{a,b}$	2.28	$89.30^{a,b,c,d}$	5.18	$99.87^{b,c,d}$	0.97	$109.98^{c,d,e}$	1.32
6 week	$78.65^{a,b}$	2.16	$87.02^{a,b,c,d}$	3.93	$94.89^{a,b,c,d}$	5.33	$109.35^{c,d,e}$	1.44
7 week	$78.34^{a,b}$	1.12	$84.37^{a,b,c,d}$	2.85	$96.05^{a,b,c,d}$	4.13	$104.70^{a,b}$	1.83
8 week	$76.09^{a,b}$	2.01	77.27^{a}	4.78	$91.00^{a,b}$	2.68	$104.96^{a,b}$	1.35
9 week	$75.62^{a,b}$	1.90	77.13^{a}	2.53	88.51^{a}	5.39	$104.80^{a,b}$	1.35
10 week	$74.42^{a,b}$	2.06	$80.77^{a,b}$	4.22	$91.64^{a,b,c}$	2.81	103.88^{a}	1.45
11 week	71.86^{a}	1.35	$80.86^{a,b}$	5.64	$91.21^{a,b}$	3.18	103.48^{a}	1.77
12 week	$74.18^{a,b}$	1.85	$82.06^{a,b,c}$	6.05	89.36^{a}	3.87	$107.07^{a,b,c,d}$	1.65
13 week	$75.01^{a,b}$	1.39	$82.39^{a,b,c}$	5.46	$92.11^{a,b,c}$	1.95	$106.25^{a,b,c}$	1.61

Table XXIII Statistical analysis of hue of sorghum Black PI Tall at different water activities. (p<0.05)

	Aw 1.0	SD	Aw 0.95	SD	Aw 0.85	SD	Aw 0.20	SD
0 day	54.93^{f}	1.33	60.17^{e}	0.98	59.60^{a}	0.89	59.45^{a}	1.45
1 day	$53.60^{e,f}$	1.48	$59.57^{d,e}$	1.05	58.80^{a}	0.92	59.45^{a}	0.70
1 week	$53.17^{d,e,,f}$	0.76	$59.03^{c,d,e}$	1.33	59.33^{a}	0.67	$60.47^{a,b,c}$	0.74
2 week	$52.63^{c,d,e,f}$	1.16	$57.97^{c,d,e}$	1.37	59.97^{a}	0.57	$61.17^{a,b,c}$	0.76
3 week	$51.67^{b,c,d,e}$	1.18	$56.47^{a,b,c,d,e}$	1.56	59.13^{a}	0.90	$60.80^{a,b,c}$	0.75
4 week	$51.97^{b,c,d,e,f}$	0.68	$56.10^{a,b,c,d}$	0.72	59.00^{a}	2.31	$61.17^{a,b,c}$	0.57
5 week	$51.53^{b,c,d,e}$	0.86	$58.03^{c,d,e}$	1.47	61.33^{a}	0.87	$61.27^{a,b,c}$	0.55
6 week	$51.53^{b,c,d,e}$	0.93	$57.33^{b,c,d,e}$	0.87	60.33^{a}	0.85	$61.53^{a,b,c}$	0.60
7 week	$51.57^{b,c,d,e}$	1.00	$55.93^{a,b,c,d}$	0.51	60.33^{a}	1.85	$60.20^{a,b}$	0.85
8 week	$50.33^{a,b,c,d}$	1.32	$53.57^{a,b}$	1.27	59.37^{a}	0.55	$60.73^{a,b,c}$	0.80
9 week	$50.07^{a,b,c,d}$	1.21	53.30^{a}	0.44	58.57^{a}	1.07	$60.80^{a,b,c}$	0.75
10 week	$49.17^{a,b}$	0.96	$55.23^{a,b,c}$	1.21	60.27^{a}	1.45	$61.30^{a,b,c}$	0.82
11 week	47.63^{a}	0.80	$55.17^{a,b,c}$	1.78	60.13^{a}	1.07	$61.17^{a,b,c}$	0.97
12 week	$49.30^{a,b}$	0.70	$56.17^{a,b,c,d}$	2.02	59.93^{a}	0.93	$62.70^{b,c}$	0.92
13 week	$49.73^{a,b,c}$	1.05	$56.37^{a,b,c,d,e}$	1.70	61.10^{a}	1.04	62.93^{a}	0.86

Table XXIV Statistical analysis of lightness of sorghum Tx430 Black at different concentrations. (p<0.05)

	0.5	SD	1	SD	5	SD
0 day	89.691^{d}	0.42	82.78^{e}	0.99	57.29^{f}	0.75
1 day	88.847^{c,d}	0.24	81.42^{d,e}	0.93	56.12^{e,f}	0.24
1 week	88.647^{c,d}	0.34	80.52^{c,d,e}	1.14	55.6^{d,e,f}	0.19
2 week	88.06^{b,c,d}	0.36	80.02^{ab,c,d,e}	0.97	55.26^{c,d,e,f}	0.06
3 week	87.62^{a,b,c,d,}	1.67	79.15^{a,b,c,d}	1.04	54.68^{b,c,d,e,f}	0.14
4 week	87.28^{a,b,c,d}	0.53	78.85^{a,b,c,d}	0.99	54.58^{a,b,c,d,e,f}	0.34
5 week	86.59^{a,b,c}	0.60	78.22^{a,b,c,d}	1.05	54.09^{a,b,c,d,e}	0.45
6 week	86.38^{a,b}	0.66	78.02^{a,b,c,d}	0.98	53.91^{a,b,c,d,e}	0.65
7 week	85.91^{a,b}	0.81	77.42^{a,b,c}	1.13	53.21^{a,b,c,d,e}	0.91
8 week	85.85^{a,b}	0.86	77.08^{a,b}	1.13	52.99^{a,b,c,d}	1.08
9 week	85.59^{a,b}	0.92	76.80^{a,b}	1.16	52.50^{a,b,c}	1.26
10 week	85.41^{a}	0.94	76.48^{a}	1.27	52.05^{a,b}	1.42
11 week	85.33^{a}	0.99	76.41^{a}	1.45	52.05^{a,b}	1.48
12 week	85.26^{a}	0.89	76.23^{a}	1.40	51.71^{a,b}	1.65
13 week	85.13^{a}	0.99	76.18^{a}	1.31	51.60^{a}	1.76

	10	SD	20	SD	40	SD
0 day	45.74^{a}	4.41	20.87^{e}	1.40	4.91^{c}	0.88
1 day	45.57^{a}	4.50	20.71^{d,e}	1.18	4.86^{c}	0.47
1 week	44.90^{a}	4.44	20.29^{c,d,e}	1.16	4.21^{b,c}	0.51
2 week	44.44^{a}	4.58	20.15^{c,d,e}	0.97	4.24^{b,c}	0.37
3 week	43.87^{a}	4.64	19.25^{b,c,d,e}	0.43	4.08^{b,c}	0.10
4 week	43.58^{a}	4.84	19.20^{b,c,d,e}	0.70	3.48^{a,b}	0.20
5 week	42.92^{a}	2.85	18.48^{a,b,c,d,e}	0.27	3.31^{a,b}	0.12
6 week	42.77^{a}	2.73	18.48^{a,b,c,d,e}	0.23	3.30^{a,b}	0.07
7 week	42.07^{a}	2.80	17.70^{a,b,c,d}	0.66	2.77^{a}	0.27
8 week	41.76^{a}	2.78	17.73^{a,b,c,d}	0.40	2.81^{a}	0.37
9 week	41.66^{a}	2.88	17.30^{a,b,c}	0.41	2.49^{a}	0.45
10 week	40.73^{a}	2.97	16.81^{a,b}	0.78	2.78^{a}	0.14
11 week	40.17^{a}	2.97	16.48^{a,b}	1.02	2.51^{a}	0.07
12 week	39.62^{a}	2.84	16.35^{a,b}	1.53	2.50^{a}	0.41
13 week	39.23^{a}	2.91	15.87^{a}	2.01	2.47^{a}	0.41

Table XXV Statistical analysis of chroma of sorghum Tx430 Black at different concentrations. (p<0.05)

	0.5	SD	1	SD	5	SD
0 day	35.36^{a}	0.97	59.26^{a}	0.42	111.97^{d}	0.32
1 day	37.83^{a,b}	0.62	64.80^{a,b}	1.27	111.85^{bd}	0.18
1 week	39.04^{a,b,c}	0.13	67.11^{a,b,c}	1.96	111.78^{c,d}	0.08
2 week	40.55^{a,b,c,d}	0.32	69.13^{a,b,c}	2.16	111.59^{b,c,d}	0.02
3 week	41.81^{a,b,c,d,e}	0.51	70.62^{b,c}	2.08	110.98^{a,b,c,d}	0.04
4 week	43.30^{a,b,c,d,e}	0.87	72.45^{b,c}	2.07	111.20^{a,b,c,d}	0.29
5 week	44.53^{b,c,d,e}	1.06	73.53^{b,c}	1.93	110.67^{a,b,c,d}	0.38
6 week	44.88^{b,c,d,e}	1.49	74.19^{b,c}	1.94	110.61^{a,b,c,d}	0.58
7 week	46.69^{c,d,e}	1.51	75.63^{b,c}	1.85	110.09^{a,b,c,d}	0.77
8 week	47.28^{c,d,e}	3.46	75.97^{b,c}	3.65	110.02^{a,b,c,d}	0.94
9 week	47.64^{c,d,e}	3.55	76.15^{c}	3.70	109.51^{a,b,c,d}	1.12
10 week	48.10^{d,e}	3.72	76.46^{c}	4.19	108.94^{a,b}	1.36
11 week	48.41^{d,e}	3.90	77.05^{c}	4.84	109.122^{a,b}	1.33
12 week	48.97^{d,e}	3.75	77.60^{c}	4.55	108.75^{a,b}	1.53
13 week	49.16^{e}	3.60	77.87^{c}	4.14	108.79^{a,b}	1.63

	10	SD	20	SD	40	SD
0 day	102.35^{a}	2.04	72.89^{c}	2.11	49.79^{d}	0.73
1 day	102.14^{a}	2.31	72.67^{b,c}	1.59	49.68^{d}	0.55
1 week	101.60^{a}	2.50	72.68^{b,c}	1.64	46.85^{b,c,d}	0.71
2 week	101.28^{a}	2.85	73.04^{c}	1.26	48.24^{c,d}	0.99
3 week	100.59^{a}	3.00	71.71^{a}	0.40	47.83^{b,c,d}	0.49
4 week	100.52^{a}	3.08	71.37^{a,b,c}	0.22	44.26^{a,b,c,d}	0.95
5 week	99.59^{a}	3.13	70.84^{a,b,c}	0.43	43.01^{a,b,c,d}	0.54
6 week	99.41^{a}	2.84	70.99^{a,b,c}	0.33	43.70^{a,b,c,d}	0.64
7 week	98.77^{a}	2.97	69.95^{a,b,c}	1.44	39.75^{a}	0.46
8 week	98.39^{a}	7.53	70.14^{a,b,c}	0.56	40.19^{a,b,c}	3.48
9 week	96.88^{a}	8.78	69.39^{a,b,c}	0.57	37.64^{a}	4.17
10 week	96.10^{a}	9.52	68.41^{a,b,c}	1.46	40.78^{a,b,c}	1.04
11 week	95.57^{a}	9.98	67.74^{a,b,c}	2.03	38.00^{a}	1.22
12 week	95.51^{a}	9.58	67.48^{a,b}	3.05	38.15^{a}	3.33
13 week	94.99^{a}	10.17	66.40^{a}	4.18	37.61^{a}	3.58

Table XXVI Statistical analysis of hue of sorghum Tx430 Black at different concentrations. (p<0.05)

	0.5	SD	1	SD	5	SD
0 day	69.70^{b}	0.17	$68.27^{f,g}$	0.12	59.93^{h}	0.57
1 day	69.63^{b}	0.12	68.43^{g}	0.12	$59.03^{g,h}$	0.25
1 week	69.37^{b}	0.29	68.03^{f}	0.06	$58.47^{f,g,h}$	0.21
2 week	$68.83^{a,b}$	0.15	67.50^{e}	0.10	$58.10^{e,f,g,h}$	0.10
3 week	68.37^{a}	0.29	67.17^{d}	0.06	$57.73^{d,e,f,g,h}$	0.15
4 week	68.23^{a}	0.32	66.83^{c}	0.06	$57.43^{c,d,e,f,g,h}$	0.23
5 week	68.07^{a}	0.35	$66.63^{b,c}$	0.06	$57.07^{b,c,d,e,f,g}$	0.31
6 week	68.03^{a}	0.21	66.50^{b}	0.00	$56.90^{a,b,c,d,e,f,g}$	0.46
7 week	68.00^{a}	0.36	66.20^{a}	0.00	$56.17^{a,b,c,d,e,f}$	0.76
8 week	68.07^{a}	0.40	66.20^{a}	0.00	$55.90^{a,b,c,d,e}$	0.90
9 week	68.00^{a}	0.44	66.00^{a}	0.10	$55.53^{a,b,c,d}$	0.95
10 week	68.07^{a}	0.38	65.97^{a}	0.06	$55.27^{a,b,c}$	1.05
11 week	68.10^{a}	0.44	66.00^{a}	0.10	$55.13^{a,b,c}$	1.20
12 week	68.23^{a}	0.32	66.03^{a}	0.15	$54.87^{a,b}$	1.31
13 week	68.20^{a}	0.26	65.97^{a}	0.15	54.70^{a}	1.35

	10	SD	20	SD	40	SD
0 day	50.43^{a}	3.45	29.50^{f}	1.30	9.67^{e}	0.35
1 day	50.33^{a}	3.55	29.40^{f}	1.14	9.63^{e}	0.60
1 week	49.67^{a}	3.53	$28.73^{e,f}$	1.10	$8.77^{d,e}$	0.45
2 week	49.23^{a}	3.62	$28.33^{d,e,f}$	0.85	$8.63^{c,d,e}$	0.42
3 week	48.77^{a}	3.67	$27.53^{c,d,e,f}$	0.51	$8.33^{c,d}$	0.15
4 week	48.33^{a}	3.82	$27.10^{b,c,d,e,f}$	0.26	$7.70^{b,c,d}$	0.17
5 week	47.95^{a}	3.84	$26.67^{a,b,c,d,e}$	0.25	$7.53^{a,b,c,d}$	0.31
6 week	47.83^{a}	3.82	$26.67^{a,b,c,d,e}$	0.25	$7.37^{a,b,c}$	0.06
7 week	47.20^{a}	4.03	$25.83^{a,b,c,d}$	0.50	$6.77^{a,b}$	0.23
8 week	46.77^{a}	4.25	$25.80^{a,b,c,d}$	0.44	$6.83^{a,b}$	0.31
9 week	46.33^{a}	4.46	$25.40^{a,b,c}$	0.44	$6.63^{a,b}$	0.45
10 week	45.97^{a}	4.67	$24.97^{a,b,c}$	0.70	$6.47^{a,b}$	0.15
11 week	45.63^{a}	4.72	$24.73^{a,b}$	0.86	$6.47^{a,b}$	0.06
12 week	45.50^{a}	4.75	$24.60^{a,b}$	1.31	6.40^{a}	0.47
13 week	45.20^{a}	4.84	24.23^{a}	1.72	6.40^{a}	0.46

Table XXVII Statistical analysis of lightness of sorghum Black PI Tall at different concentrations. ($p<0.05$)

	0.1	SD	0.5	SD	1	SD	5	SD	10	SD	20	SD
0 day	93.18^{a}	0.23	74.85^{a}	0.25	$62.34^{a,b}$	2.95	34.52^{f}	1.81	16.21^{i}	1.03	4.70^{g}	0.89
1 day	92.82^{a}	0.16	74.28^{a}	0.22	60.27^{a}	0.23	$32.97^{e,f}$	1.57	$14.48^{h,i}$	0.31	2.76^{f}	0.10
1 week	93.39^{a}	0.11	74.44^{a}	0.53	60.17^{a}	0.44	$32.32^{d,e,f}$	1.49	$13.28^{g,h}$	0.91	$2.52^{e,f}$	0.14
2 week	92.77^{a}	0.24	73.55^{a}	0.66	59.40^{a}	0.62	$31.32^{c,d,e,f}$	1.42	$12.32^{f,g,h}$	0.67	$2.27^{d,e,f}$	0.15
3 week	93.17^{a}	0.42	73.56^{a}	0.38	$58.82^{a,b}$	0.83	$30.58^{b,c,d,e}$	1.31	$11.47^{e,f,g}$	0.64	$2.05^{c,d,e,f}$	0.15
4 week	93.53^{a}	0.69	73.35^{a}	0.39	$58.23^{a,b}$	0.98	$30.19^{a,b,c,d,e}$	1.25	$10.85^{d,e,f,g}$	0.59	$1.91^{c,d,e}$	0.17
5 week	93.36^{a}	0.71	73.18^{a}	0.50	$58.57^{a,b}$	0.59	$29.83^{a,b,c,d,e}$	1.22	$10.41^{d,e,f}$	0.61	$1.78^{b,c,d,e}$	0.15
6 week	93.18^{a}	0.90	72.97^{a}	0.63	$58.00^{a,b}$	1.02	$29.39^{a,b,c,d}$	0.98	$9.84^{c,d,e,f}$	0.54	$1.67^{a,b,c,d}$	0.15
7 week	93.40^{a}	1.08	73.01^{a}	0.85	$57.65^{a,b}$	1.00	$29.01^{a,b,c,d}$	0.74	$9.28^{b,c,d,e}$	0.64	$1.52^{a,b,c,d}$	0.15
8 week	93.64^{a}	1.00	72.87^{a}	0.92	$57.36^{a,b}$	1.06	$28.75^{a,b,c}$	0.52	$8.84^{a,b,c,d,e}$	0.78	$1.41^{a,b,c}$	0.13
9 week	93.84^{a}	1.08	72.94^{a}	0.99	$57.36^{a,b}$	1.16	$28.59^{a,b,c}$	0.43	$8.56^{a,b,c,d}$	0.81	$1.37^{a,b,c}$	0.12
10 week	94.24^{a}	1.48	72.94^{a}	1.36	$56.70^{a,b}$	0.99	$27.83^{a,b}$	0.27	$7.21^{a,b,c}$	1.10	$1.07^{a,b}$	0.14
11 week	94.44^{a}	1.59	72.90^{a}	1.59	53.43^{a}	6.20	$27.64^{a,b}$	0.61	$6.89^{a,b}$	1.22	$1.03^{a,b}$	0.15
12 week	93.86^{a}	1.82	72.50^{a}	1.64	$56.44^{a,b}$	1.11	$27.27^{a,b}$	0.76	$6.59^{a,b}$	1.30	0.98^{a}	0.12
13 week	94.16^{a}	1.96	72.69^{a}	1.81	$56.63^{a,b}$	1.13	27.18^{a}	1.23	6.19^{a}	1.51	0.90^{a}	0.12

Table XXVIII Statistical analysis of chroma of sorghum Black PI Tall at different concentrations. ($p<0.05$)

	0.1	SD	0.5	SD	1	SD	5	SD	10	SD	20	SD
0 day	20.63^{a}	0.62	82.96^{a}	0.28	112.06^{d,e,f}	3.86	91.34^{f}	2.05	69.31^{f}	1.36	51.88^{j}	2.86
1 day	22.03^{a}	0.72	85.04^{a}	0.51	114.43^{f}	0.57	89.79^{e,f}	1.80	67.50^{e,f}	0.60	43.06^{i}	0.65
1 week	21.69^{a}	0.24	84.10^{a}	0.42	113.94^{e,f}	0.35	89.55^{d,e,f}	1.65	66.70^{e,f}	1.80	41.88^{h,i}	0.85
2 week	21.43^{a}	0.59	83.21^{a}	0.95	112.80^{d,e,f}	0.40	88.39^{c,d,e,f}	1.52	66.27^{e,f}	0.96	39.71^{g,h,i}	1.08
3 week	20.98^{a}	1.10	83.04^{a}	1.04	119.91^{d,e,f}	0.72	87.54^{b,c,d,e}	1.35	65.54^{d,e,f}	0.99	37.82^{f,g,h}	1.48
4 week	20.63^{a}	1.54	82.91^{a}	1.41	111.70^{c,d,e,f}	0.48	87.18^{a,b,c,d,e}	1.31	65.06^{c,d,e,f}	0.88	36.54^{e,f,g}	1.68
5 week	20.44^{a}	1.74	82.38^{a}	1.55	111.37^{b,c,d,e,f}	0.43	86.79^{a,b,c,d,e}	1.19	64.56^{c,d,e,f}	0.85	35.24^{d,e,f,g}	1.61
6 week	20.24^{a}	2.18	81.74^{a}	1.93	110.81^{a,b,c,d,e}	0.38	86.31^{a,b,c,d,e}	1.02	64.23^{b,c,d,e}	0.74	34.16^{d,e,f}	1.91
7 week	19.70^{a}	2.40	81.20^{a}	2.46	110.30^{a,b,c,d}	0.38	85.92^{a,b,c,d}	0.76	63.64^{b,c,d,e}	0.76	32.37^{d,e}	1.03
8 week	19.60^{a}	2.58	80.74^{a}	2.65	110.17^{a,b,c,d}	0.89	85.52^{a,b,c}	0.54	63.07^{a,b,c,d,e}	0.95	31.56^{c,d,e}	1.42
9 week	19.56^{a}	2.78	80.55^{a}	2.80	109.69^{a,b,c,d}	0.44	85.35^{a,b,c}	0.45	62.84^{a,b,c,d,e}	0.93	30.44^{b,c,d}	1.75
10 week	19.28^{a}	3.90	79.95^{a}	4.07	108.43^{a,b,c}	0.19	84.50^{a,b}	0.34	60.97^{a,b,c,d}	1.86	26.49^{a,b,c}	2.03
11 week	19.35^{a}	4.23	80.06^{a}	4.57	108.33^{a,b}	0.32	84.36^{a,b}	0.65	60.49^{a,b,c}	2.28	26.00^{a,b}	2.00
12 week	19.50^{a}	4.49	79.71^{a}	4.71	107.59^{a}	0.34	83.56^{a}	0.82	59.49^{a,b}	2.73	24.99^{a}	1.77
13 week	19.69^{a}	5.11	79.95^{a}	5.30	107.66^{a}	0.26	83.54^{a}	1.34	58.46^{a}	3.71	24.18^{a}	1.82

Table XXIX Statistical analysis of hue of sorghum Black PI Tall at different concentrations. ($p<0.05$)

	0.1	SD	0.5	SD	1	SD	5	SD	10	SD	20	SD
0 day	64.47^{a}	0.12	64.57^{a}	0.12	61.93^{d}	1.10	40.60^{e}	1.49	23.70^{i}	1.08	8.87^{h}	1.19
1 day	64.40^{a}	0.10	64.60^{a}	0.10	$61.20^{c,d}$	0.10	$39.23^{d,e}$	1.29	$21.60^{h,i}$	0.30	6.30^{g}	0.10
1 week	$65.37^{a,b}$	0.67	64.23^{a}	0.06	$60.97^{b,c,d}$	0.15	$38.40^{c,d,e}$	1.23	$19.93^{g,h}$	0.90	$5.87^{f,g}$	0.15
2 week	$65.87^{a,b,c}$	0.93	64.03^{a}	0.12	$60.67^{a,b,c,d}$	0.32	$37.60^{b,c,d}$	1.23	$18.57^{f,g,h}$	0.76	$5.57^{e,f,g}$	0.21
3 week	$66.27^{a,b,c}$	1.07	63.93^{a}	0.21	$60.47^{a,b,c,d}$	0.42	$39.97^{a,b,c,d}$	1.17	$17.47^{e,f,g}$	0.76	$5.30^{d,e,f,g}$	0.20
4 week	$67.83^{a,b,c}$	1.40	64.03^{a}	0.31	$60.00^{a,b,c}$	0.52	$36.57^{a,b,c,d}$	1.10	$16.63^{d,e,f,g}$	0.76	$5.07^{d,e,f}$	0.21
5 week	$67.70^{a,b,c}$	1.45	63.87^{a}	0.32	$60.13^{a,b,c}$	0.57	$36.27^{a,b,c}$	1.00	$16.07^{d,e,f}$	0.80	$4.93^{c,d,e,f}$	0.21
6 week	$68.77^{a,b,c}$	1.72	64.00^{a}	0.36	$59.93^{a,b,c}$	0.67	$35.90^{a,b,c}$	0.89	$15.23^{c,d,e,f}$	0.68	$4.80^{b,c,d,e,f}$	0.17
7 week	$69.07^{a,b,c}$	1.84	63.87^{a}	0.49	$59.70^{a,b,c}$	0.56	$35.53^{a,b,c}$	0.68	$14.47^{b,c,d,e}$	0.91	$4.60^{a,b,c,d,e}$	0.17
8 week	$69.17^{a,b,c}$	2.06	63.87^{a}	0.49	$59.53^{a,b,c}$	0.60	$35.37^{a,b}$	0.47	$13.90^{a,b,c,d}$	1.01	$4.33^{a,b,c,d}$	0.23
9 week	$69.73^{a,b,c}$	2.41	63.83^{a}	0.55	$59.53^{a,b,c}$	0.70	$35.23^{a,b}$	0.42	$13.47^{a,b,c,d}$	1.12	$4.37^{a,b,c,d}$	0.21
10 week	$71.63^{b,c}$	3.42	64.07^{a}	0.58	$59.23^{a,b}$	0.55	34.60^{a}	0.26	$11.87^{a,b,c}$	1.62	$3.93^{a,b,c}$	0.23
11 week	$71.57^{b,c}$	3.55	64.10^{a}	0.70	$59.93^{a,b,c}$	0.65	34.67^{a}	0.59	$11.20^{a,b}$	1.65	$3.87^{a,b,c}$	0.21
12 week	72.50^{c}	3.80	64.17^{a}	0.67	59.17^{a}	0.60	34.20^{a}	0.70	10.87^{a}	1.79	$3.83^{a,b}$	0.15
13 week	72.57^{c}	4.01	64.27^{a}	0.68	59.20^{a}	0.66	34.10^{a}	1.15	10.40^{a}	2.00	3.60^{a}	0.20

APPENDIX B

% COLOR RETENTION

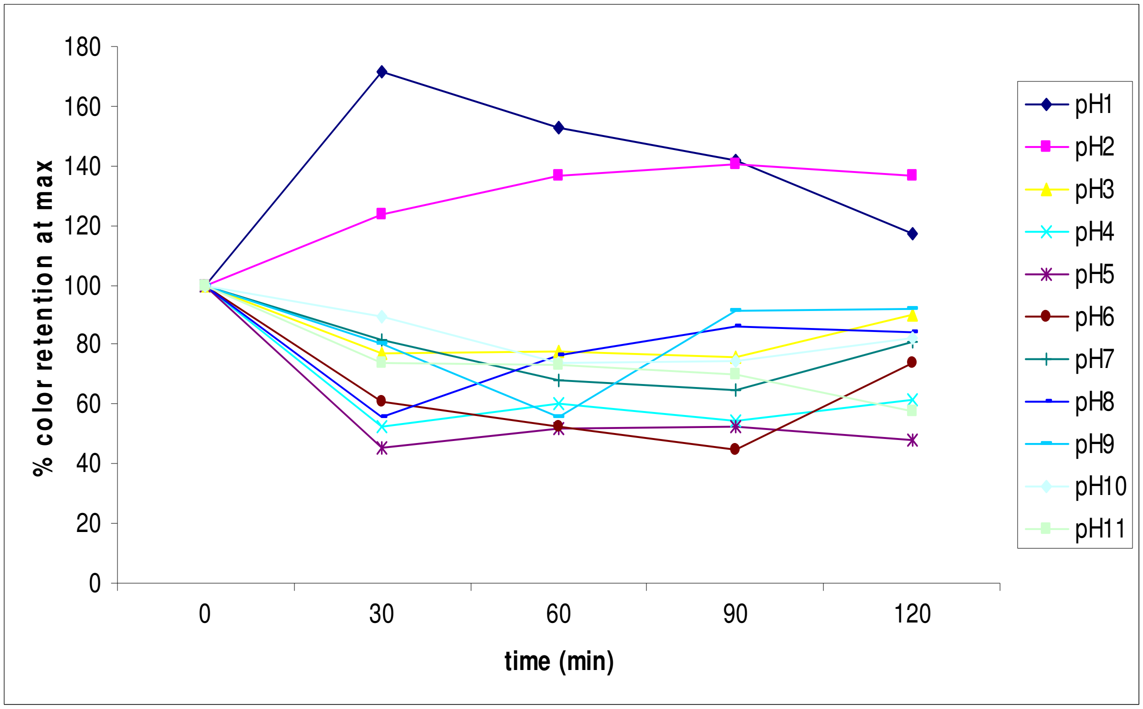

Fig. 58. % Color retention of Tx430 Black sorghum bran extracts at different pHs.

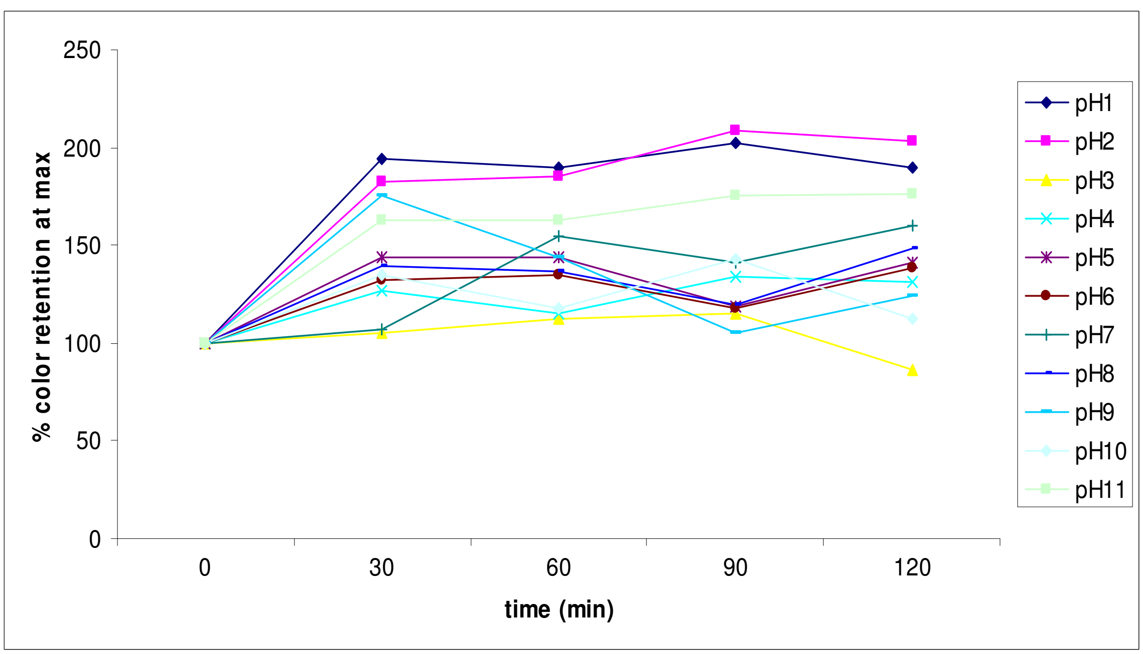

Fig. 59. % Color retention of Black PI Tall sorghum bran extracts at different pHs.

APPENDIX C

COLOR COMPARISON OF SORGHUM BRAN EXTRACTS AND STANDARDS

RED N0. 3 AND RED NO. 40

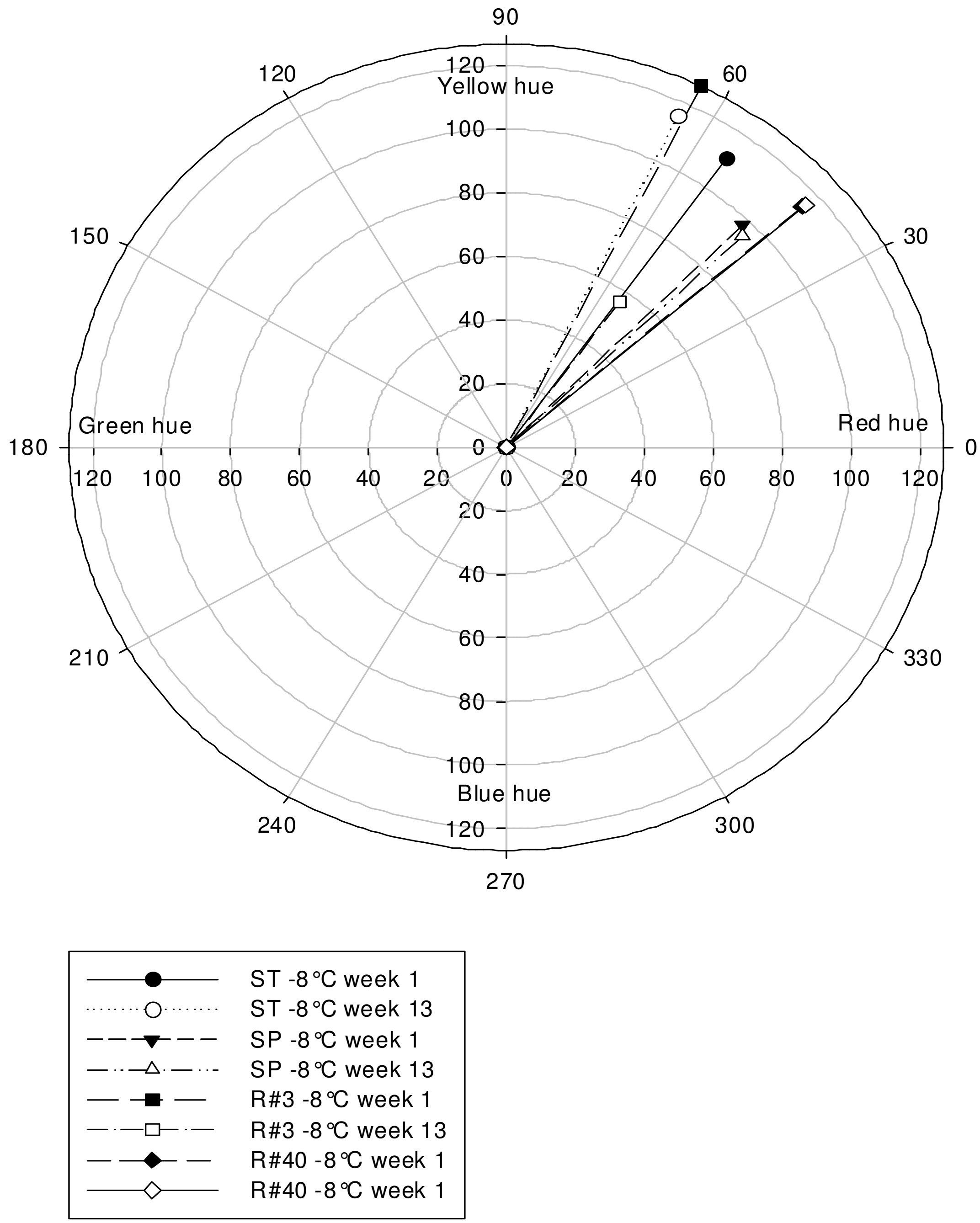

Fig. 60. Changes in color attributes of Tx430 Black (ST) and Black PI Tall (BTS) sorghum bran extracts and standard Red No. 3(R#3) and Red No. 40(R#40) at -8°C, after 1 and 13 weeks. Plot represents the chroma and the hue.

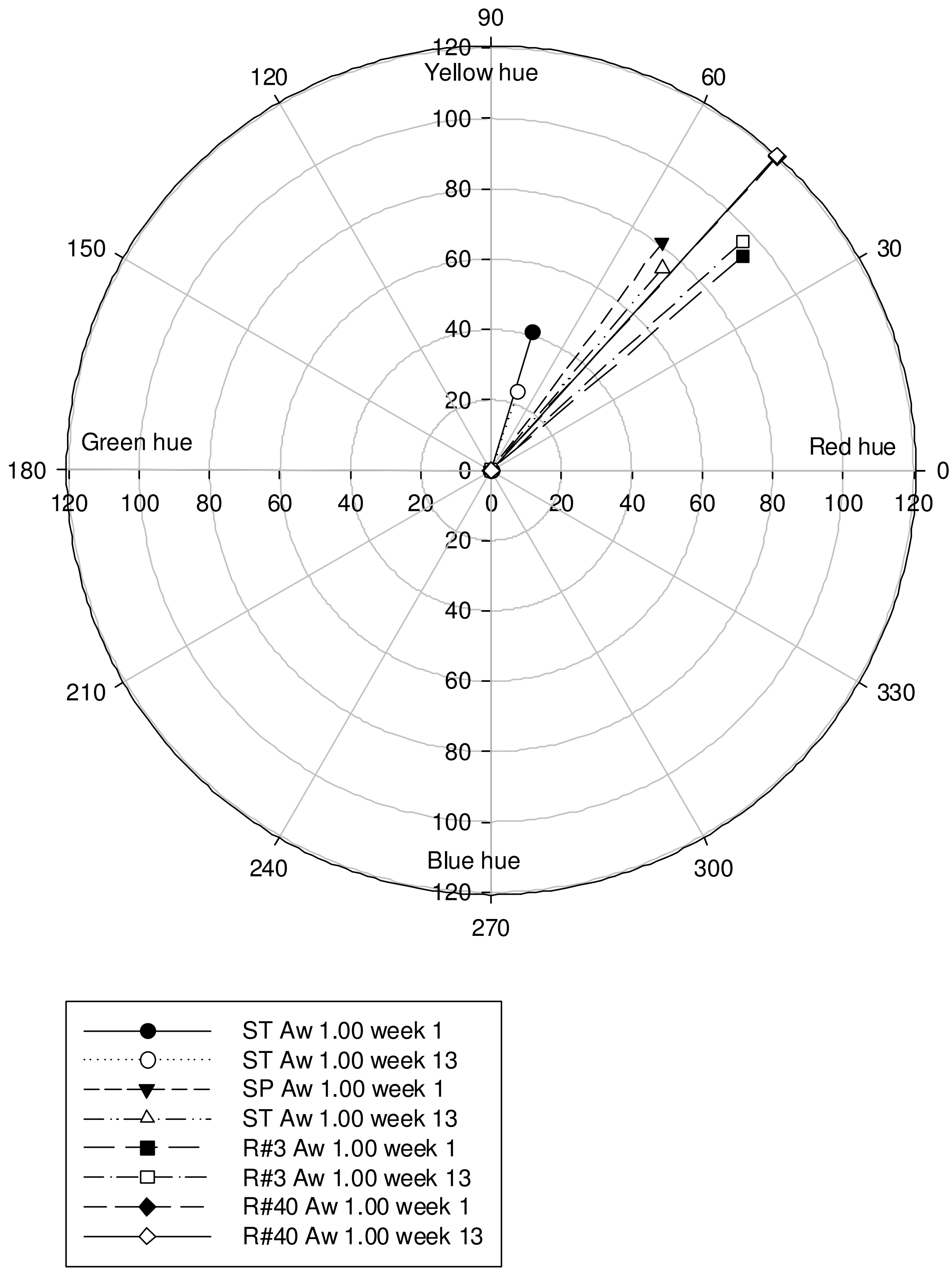

Fig. 61. Changes in color attributes of Tx430 Black (ST) and Black PI Tall (BTS) sorghum bran extracts and standard Red No. 3(R#3) and Red No. 40(R#40) at Aw 1.00, after 1 and 13 weeks. Plot represents the chroma and the hue.

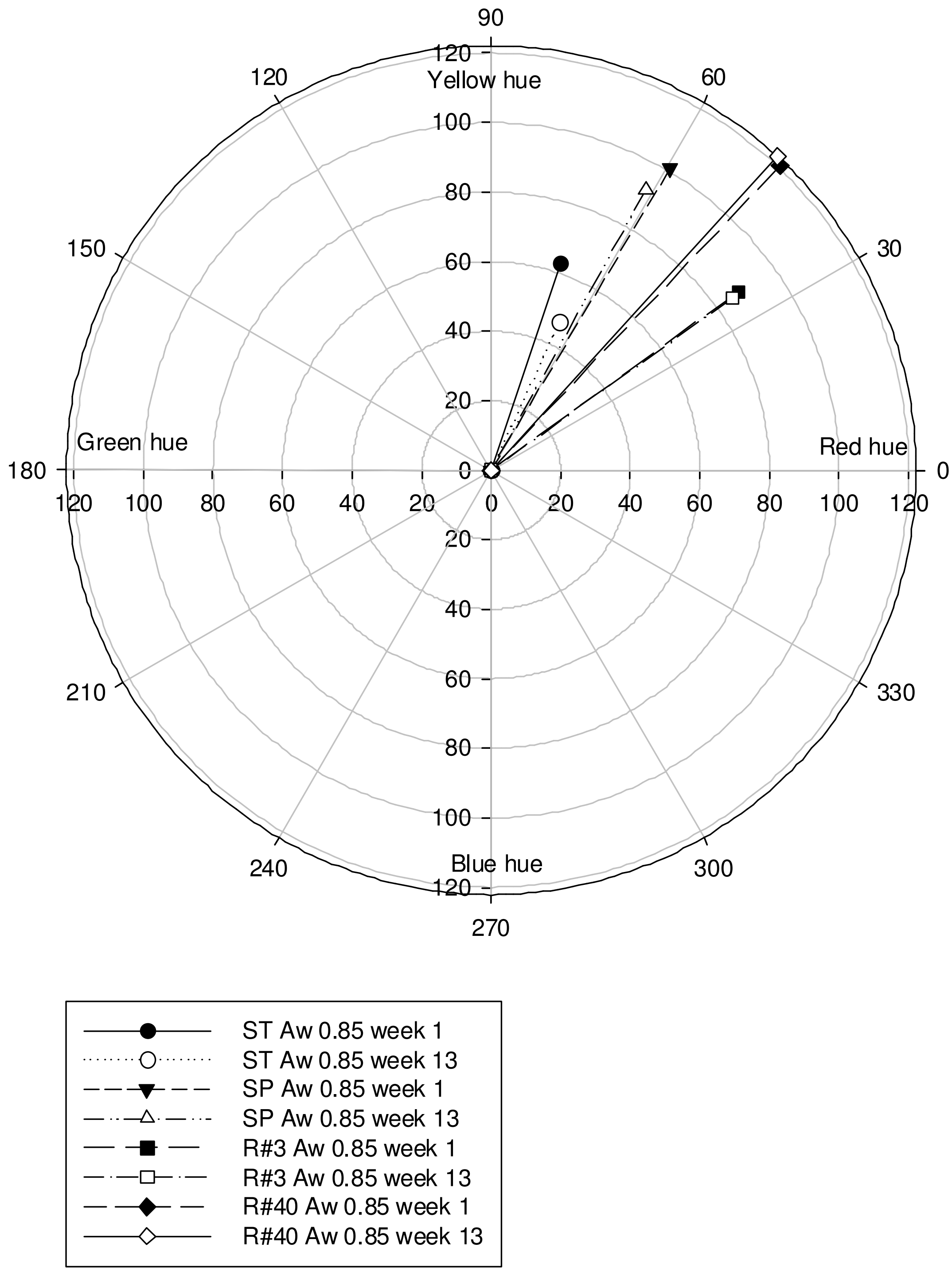

Fig. 62. Changes in color attributes of Tx430 Black (ST) and Black PI Tall (BTS) sorghum bran extracts and standard Red No. 3(R#3) and Red No. 40(R#40) at Aw 0.85, after 1 and 13 weeks. Plot represents the chroma and the hue.

APPENDIX D

COLOR STABILITY AFTER 1 YEAR

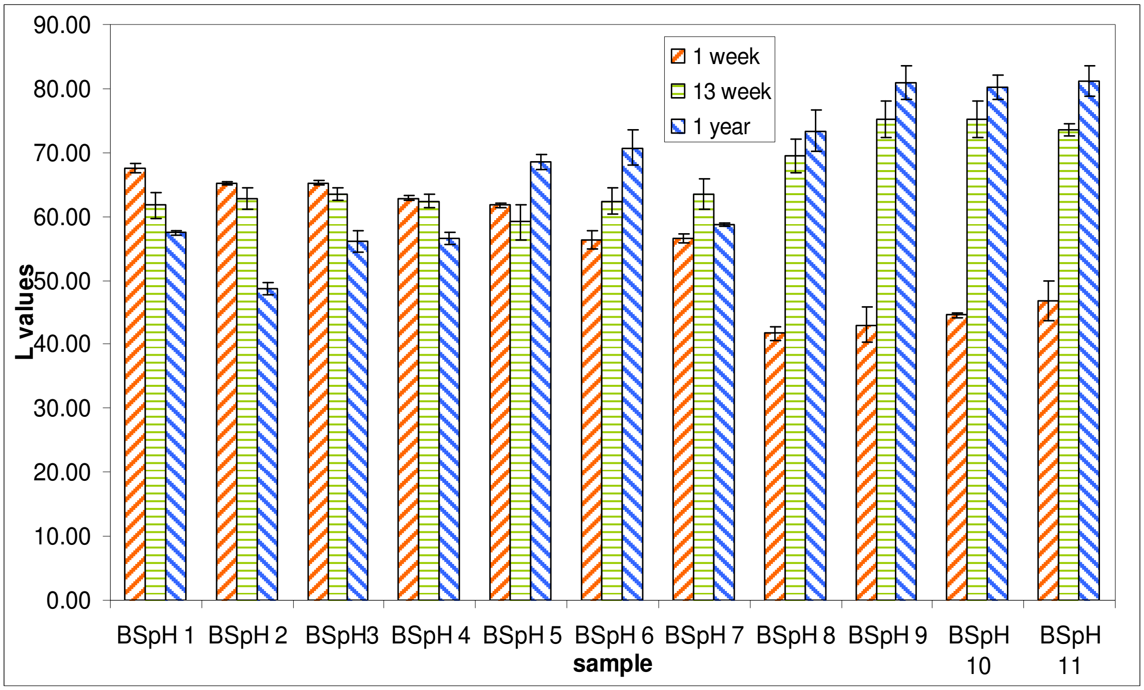

Fig. 63. Lightness values of Tx430 Black sorghum bran extracts (BS) at 1 and 13 weeks, and 1 year at different pHs (P< 0.05).

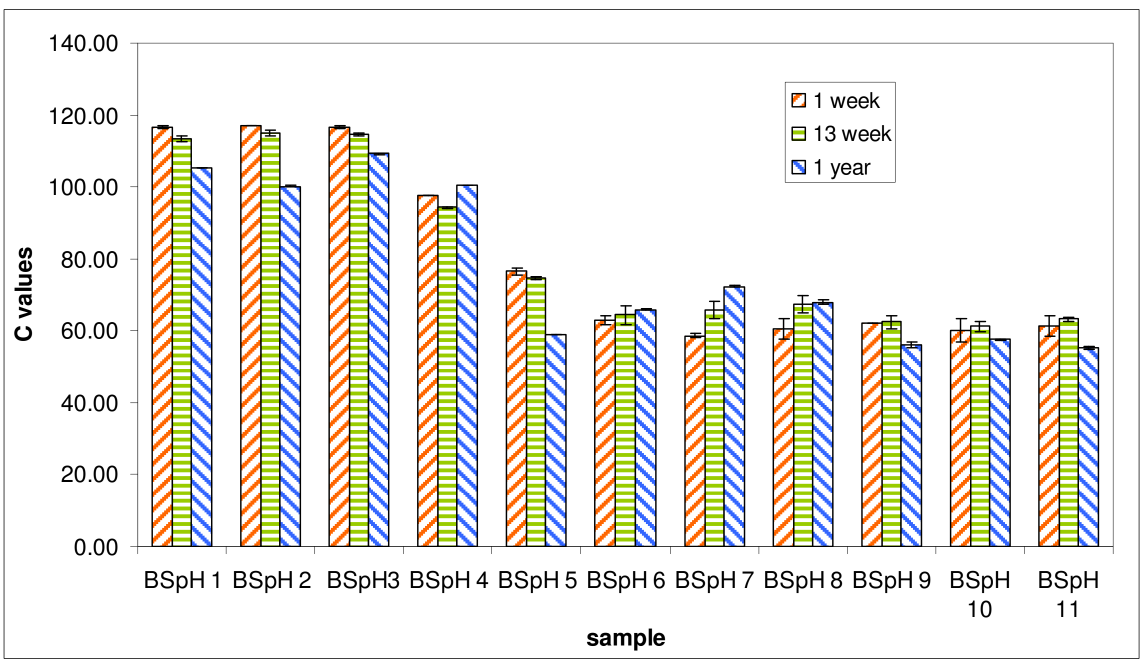

Fig. 64. Chroma values of Tx430 Black sorghum bran extracts (BS) at 1 and 13 weeks, and 1 year at different pHs (P< 0.05).

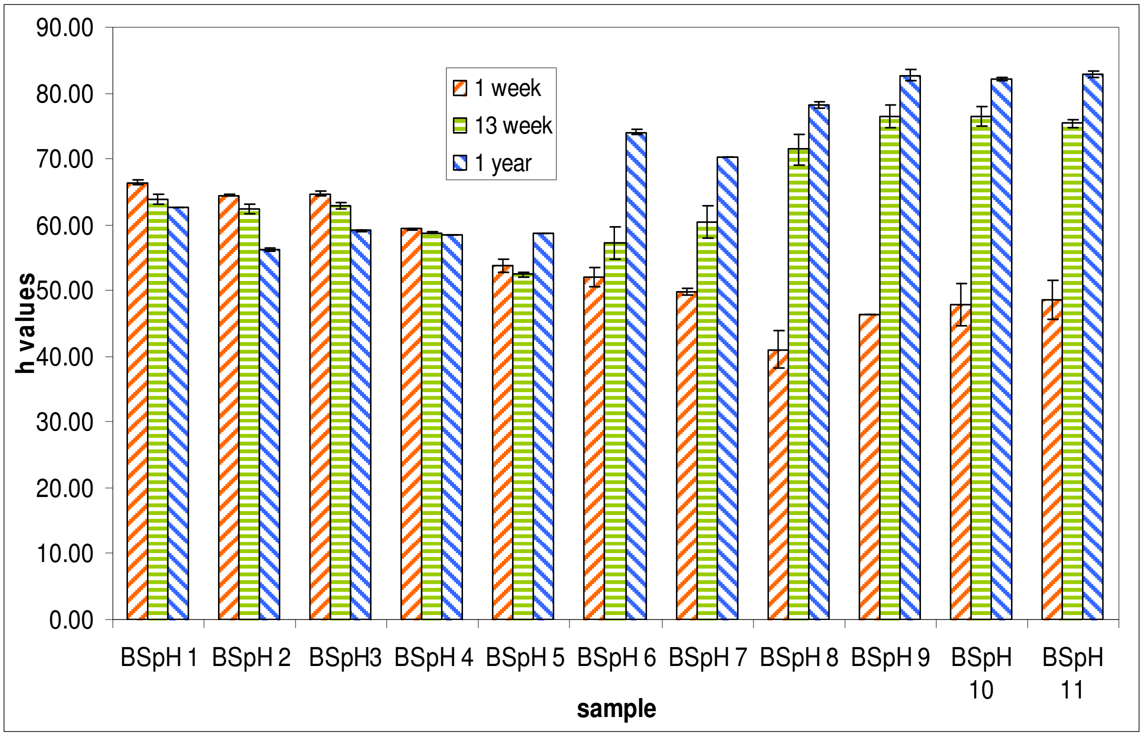

Fig. 65. Hue values of Tx430 Black sorghum bran extracts (BS) at 1 and 13 weeks, and 1 year at different pHs (P< 0.05).

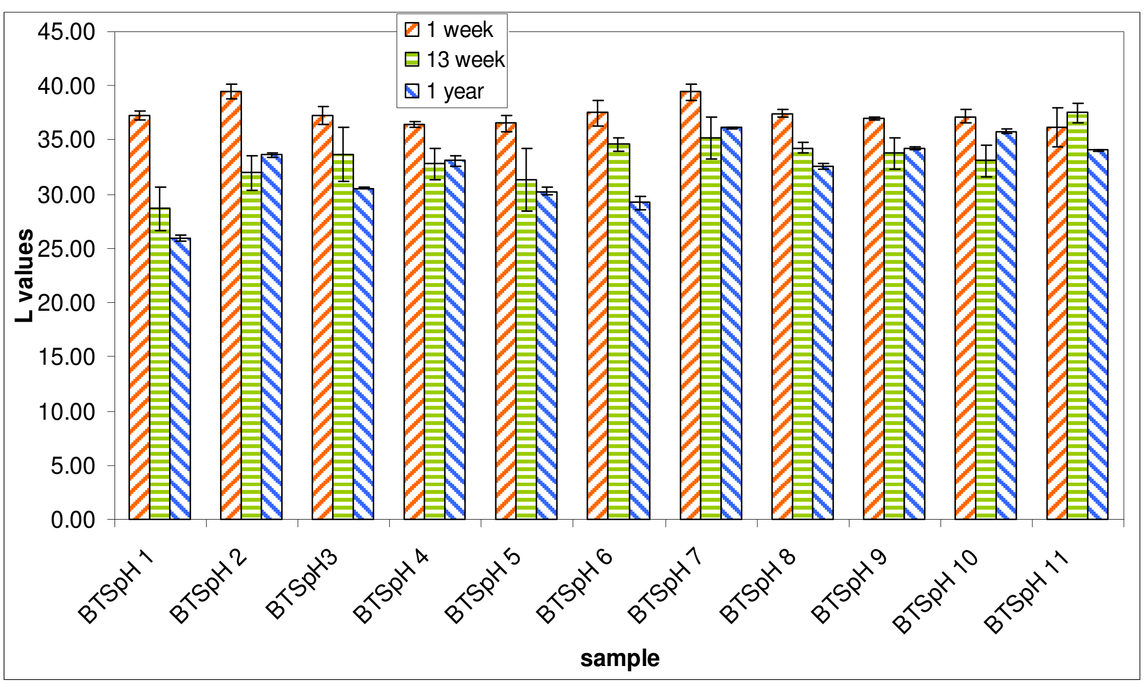

Fig. 66. Lightness values of Black PI Tall sorghum bran extracts (BTS) at 1 and 13 weeks, and 1 year at different pHs (P< 0.05).

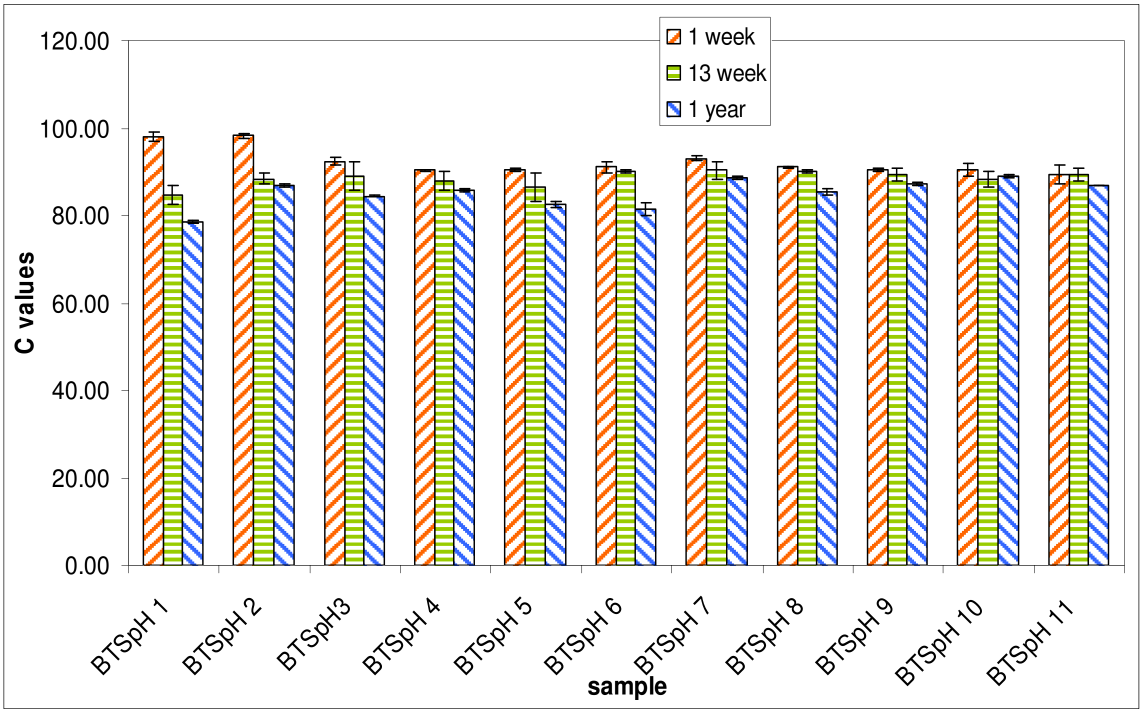

Fig. 67. Chroma values of Black PI Tall sorghum bran extracts (BTS) at 1 and 13 weeks, and 1 year at different pHs (P< 0.05).

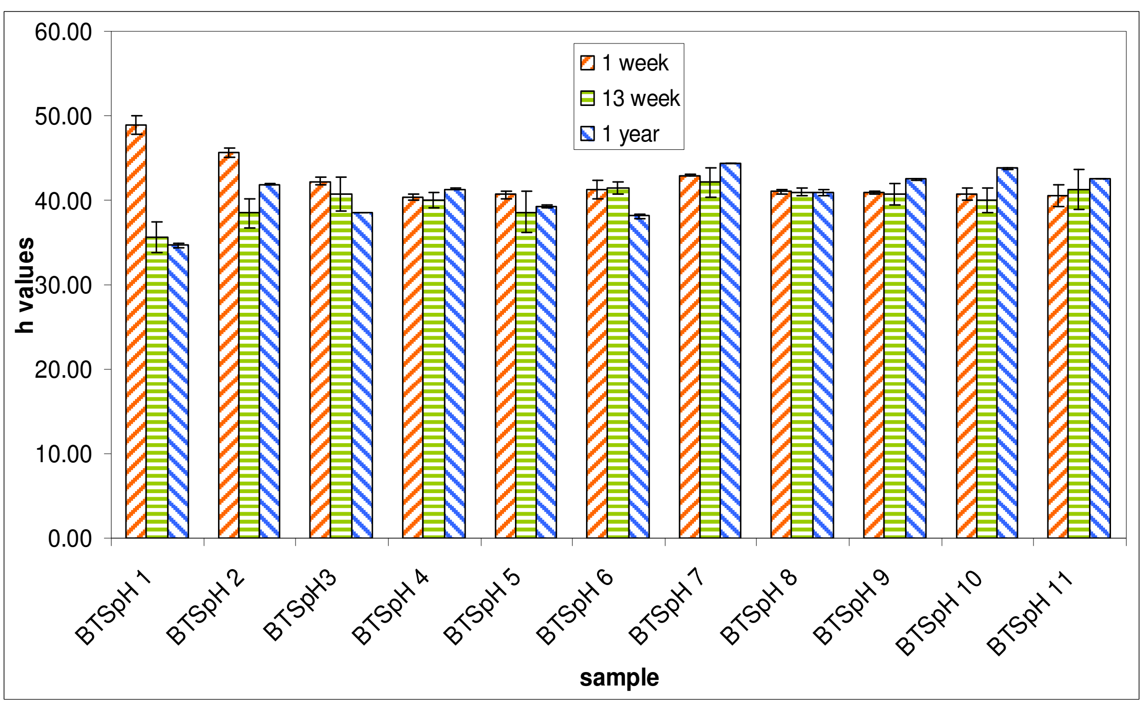

Fig. 68. Hue values of Black PI Tall sorghum bran extracts (BTS) at 1 and 13 weeks, and 1 year at different pHs (P< 0.05).

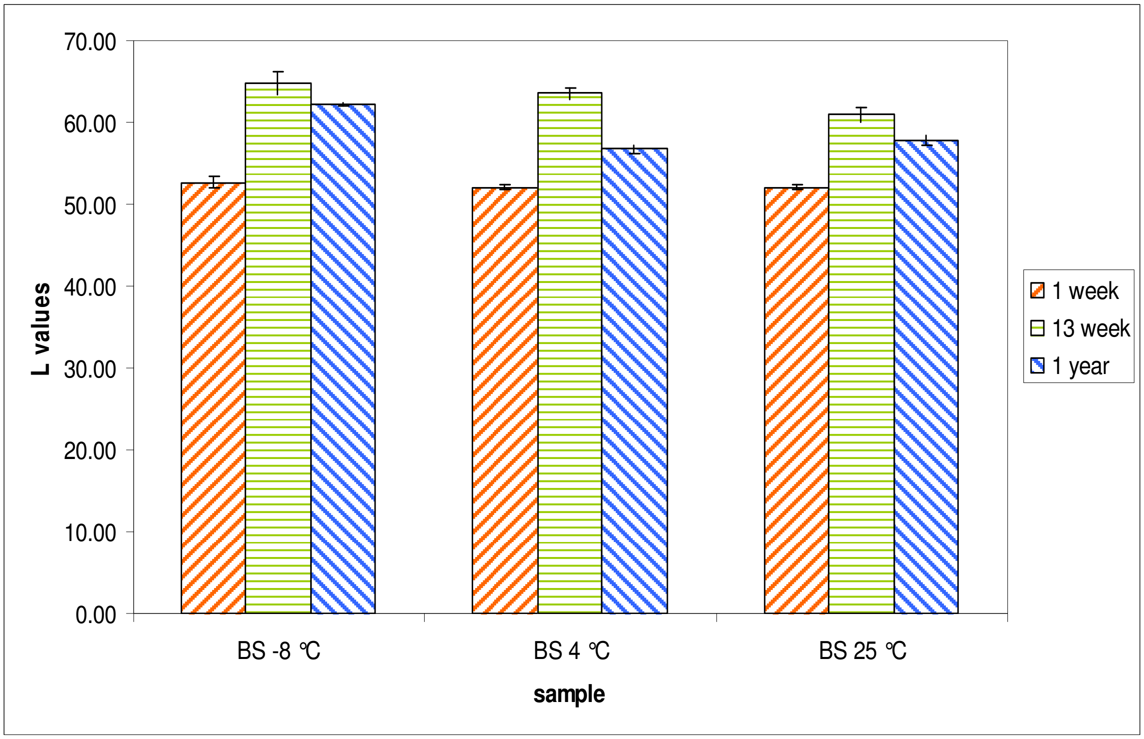

Fig. 69. Lightness values of Tx430 Black sorghum bran extracts (BS) at 1 and 13 weeks, and 1 year at different temperatures (P< 0.05).

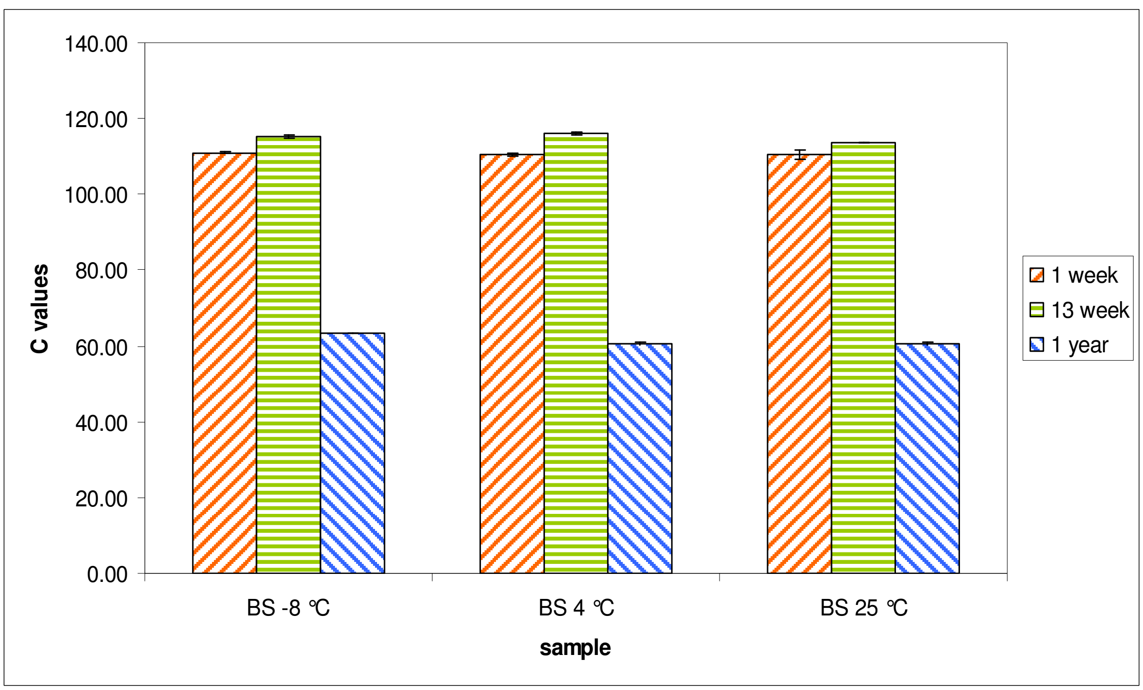

Fig. 70. Chroma values of Tx430 Black sorghum bran extracts (BS) at 1 and 13 weeks, and 1 year at different temperatures (P< 0.05).

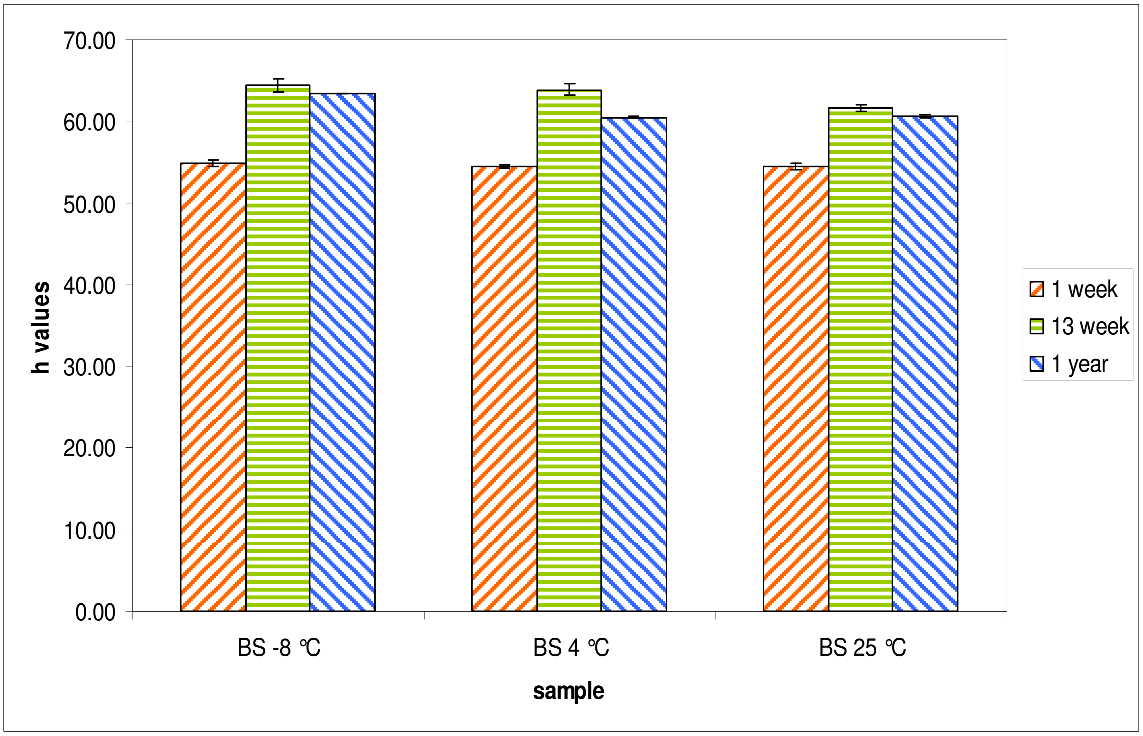

Fig. 71. Hue values of Tx430 Black sorghum bran extracts (BS) at 1 and 13 weeks, and 1 year at different temperatures (P< 0.05).

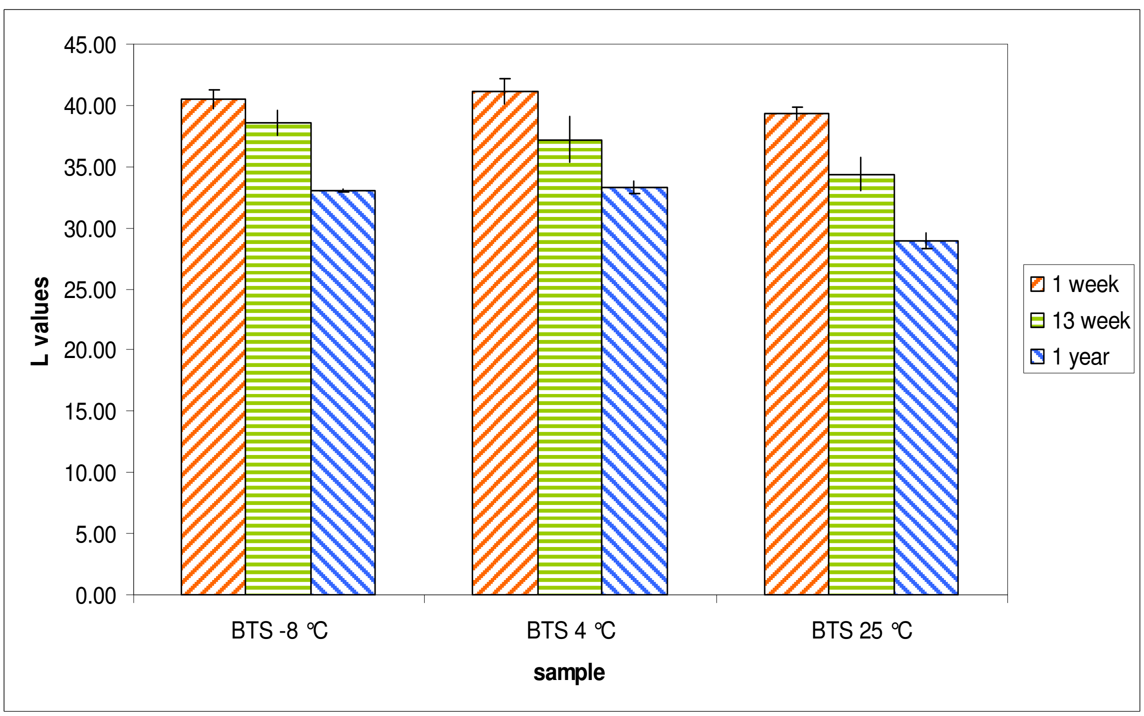

Fig. 72. Lightness values of Black PI Tall sorghum bran extracts (BTS) at 1 and 13 weeks, and 1 year at different temperatures (P< 0.05).

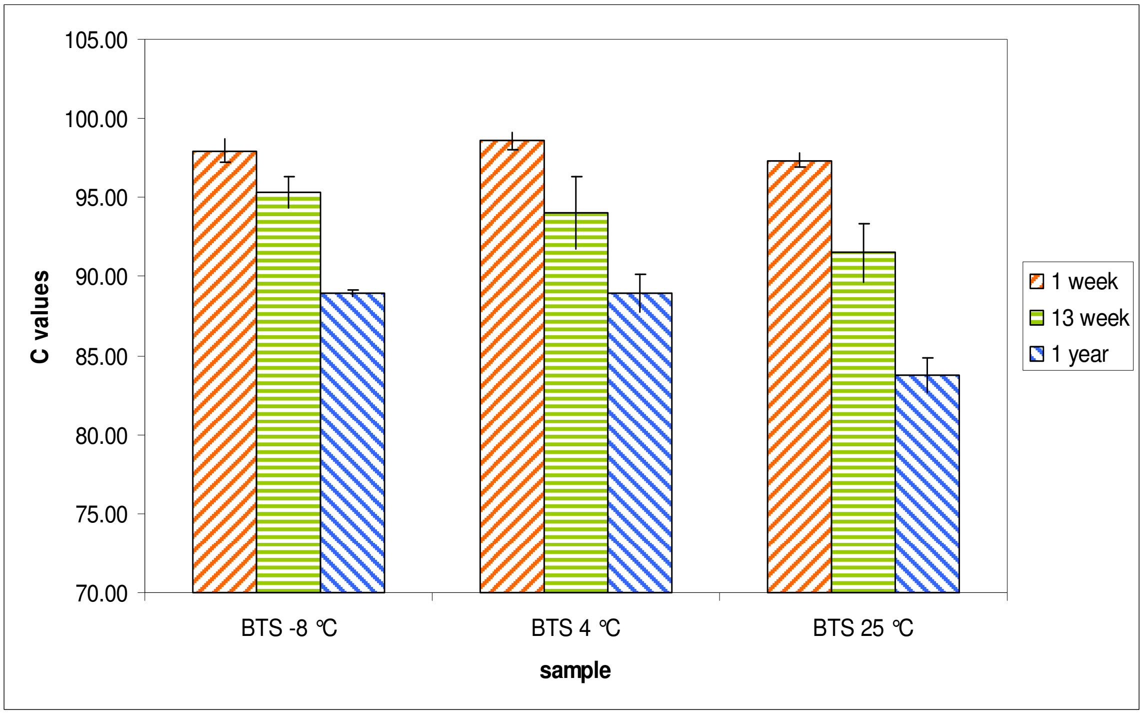

Fig. 73. Chroma values of Black PI Tall sorghum bran extracts (BTS) at 1 and 13 weeks, and 1 year at different temperatures (P< 0.05).

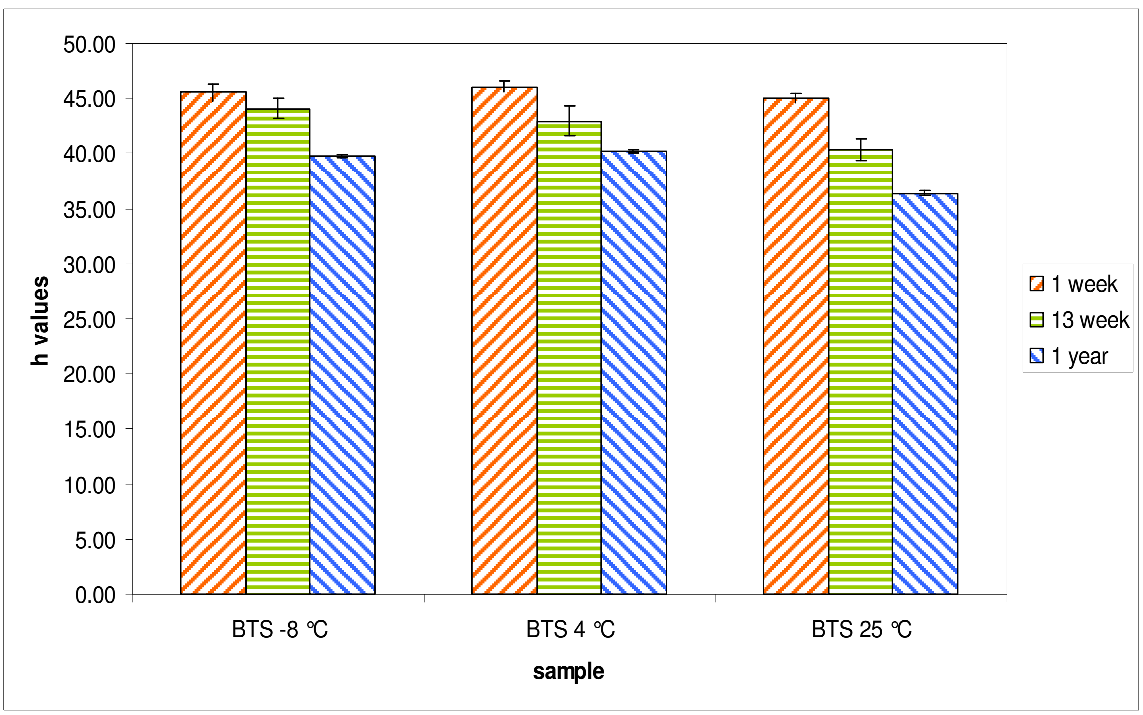

Fig. 74. Hue values of Black PI Tall sorghum bran extracts (BTS) at 1 and 13 weeks, and 1 year at different temperatures (P< 0.05).

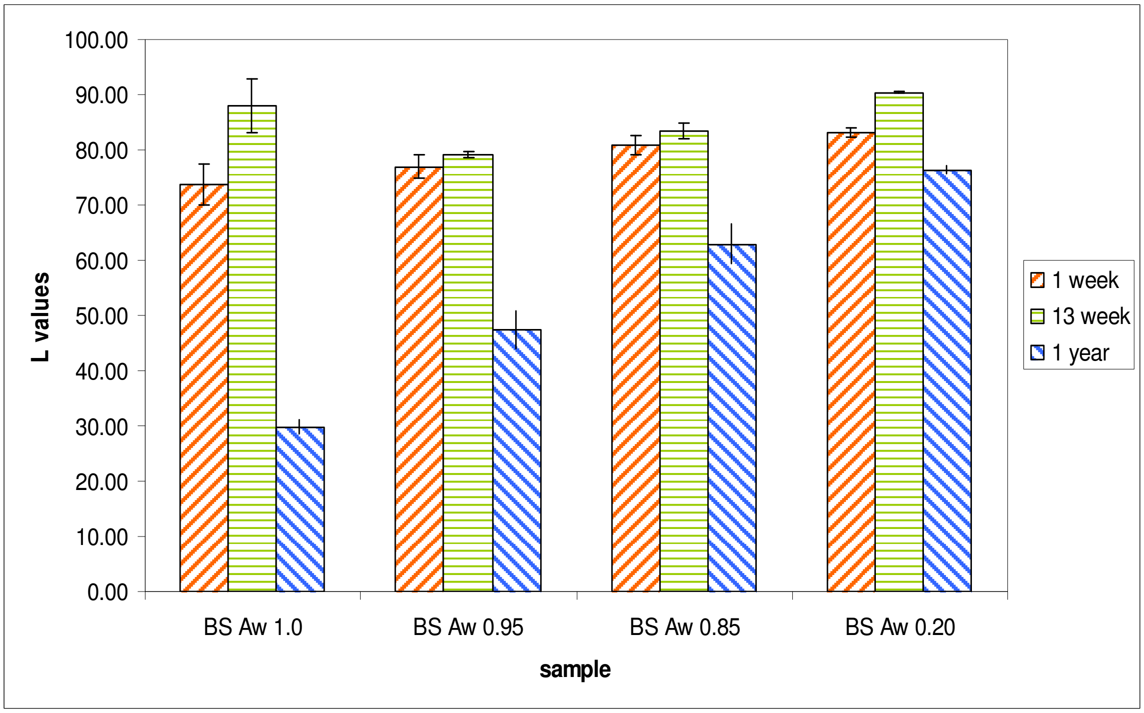

Fig. 75. Lightness values of Tx430 Black sorghum bran extracts (BS) at 1 and 13 weeks, and 1 year at different Aw (P< 0.05).

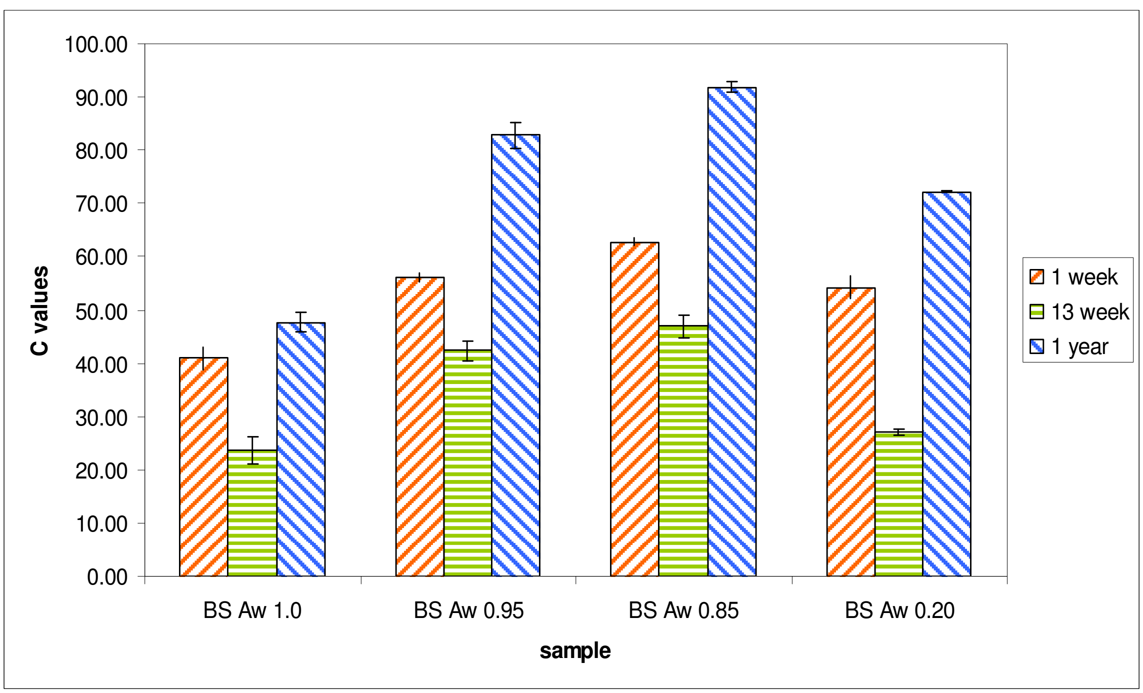

Fig. 76. Chroma values of Tx430 Black sorghum bran extracts (BS) at 1 and 13 weeks, and 1 year at different Aw (P< 0.05).

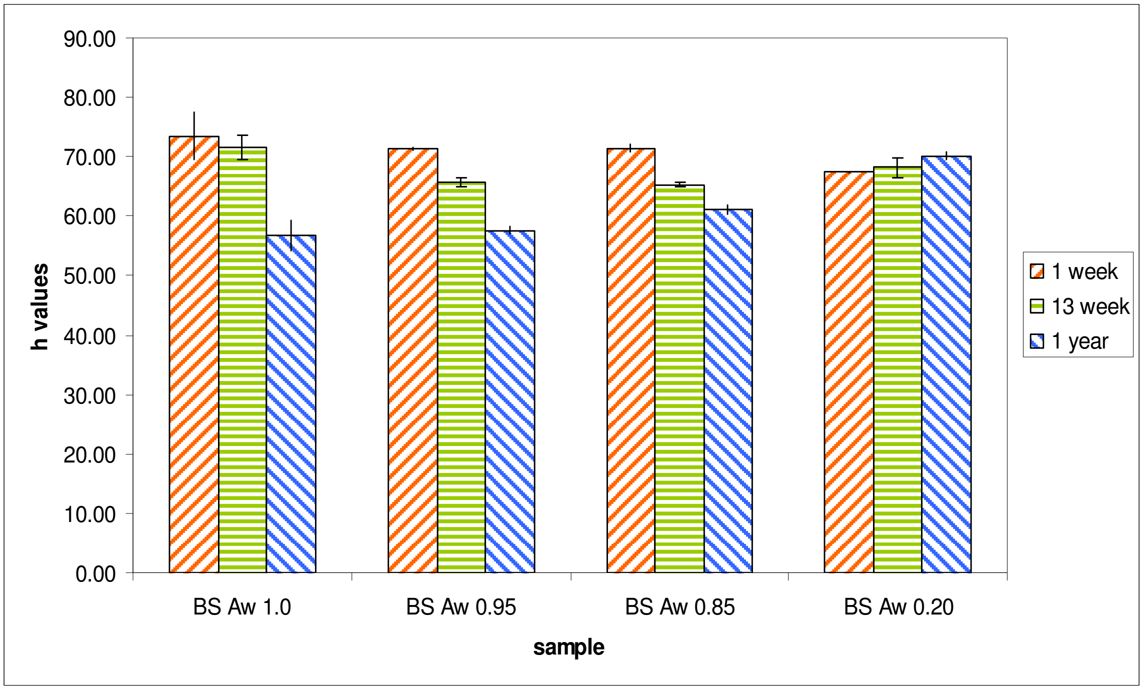

Fig. 77. Hue values of Tx430 Black sorghum bran extracts (BS) at 1 and 13 weeks, and 1 year at different Aw (P< 0.05).

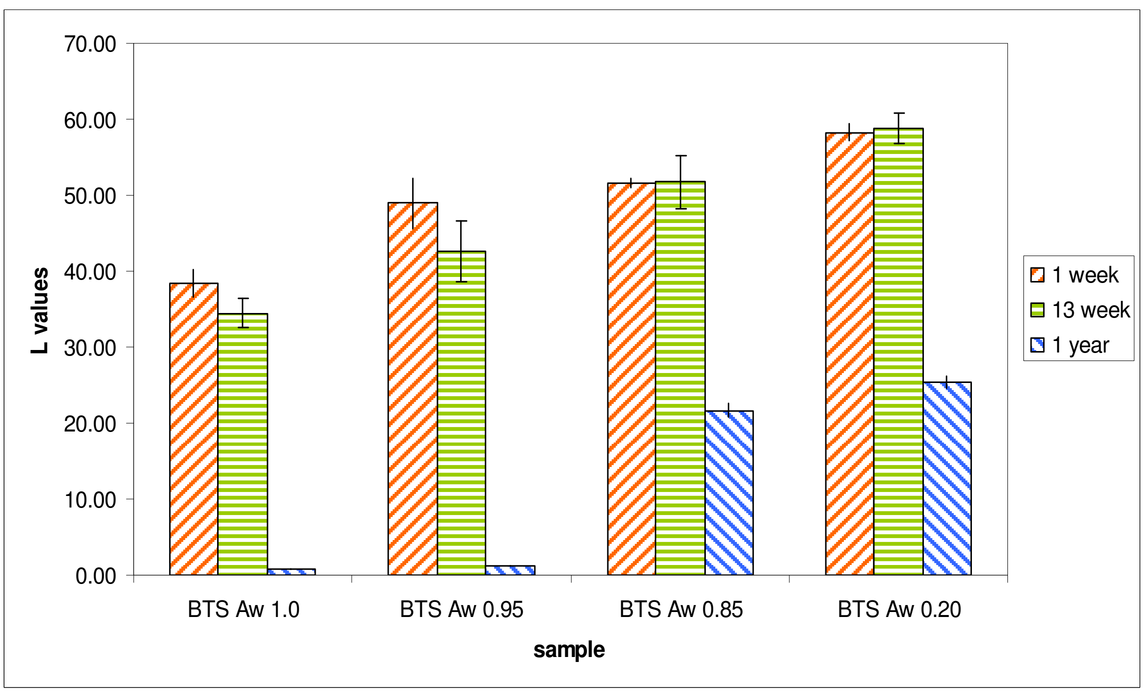

Fig. 78. Lightness values of Black PI Tall sorghum bran extracts (BTS) at 1 and 13 weeks, and 1 year at different Aw (P< 0.05).

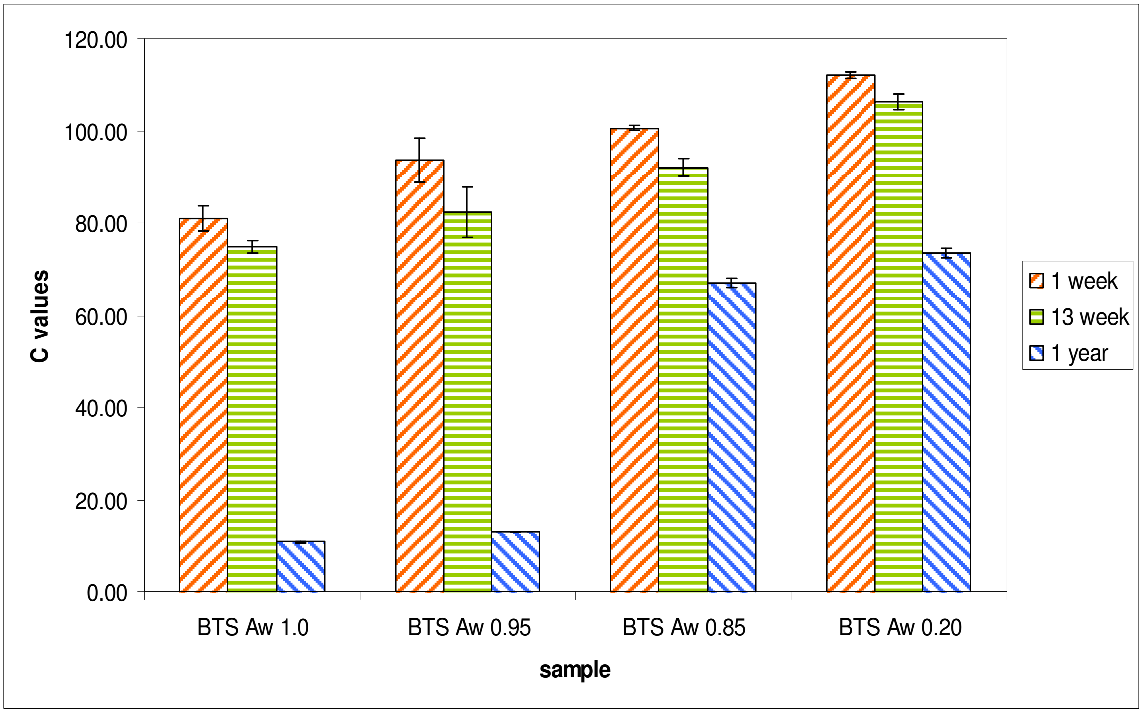

Fig. 79. Chroma values of Black PI Tall sorghum bran extracts (BTS) at 1 and 13 weeks, and 1 year at different Aw (P< 0.05).

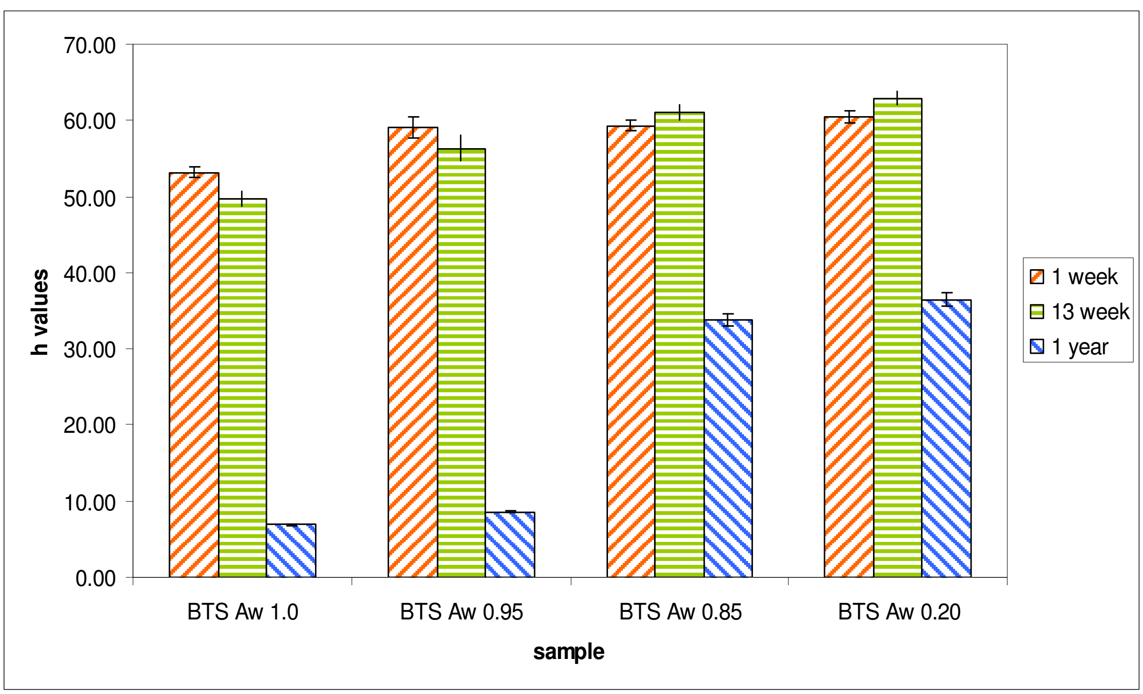

Fig. 80. Hue values of Black PI Tall sorghum bran extracts (BTS) at 1 and 13 weeks, and 1 year at different Aw (P< 0.05).

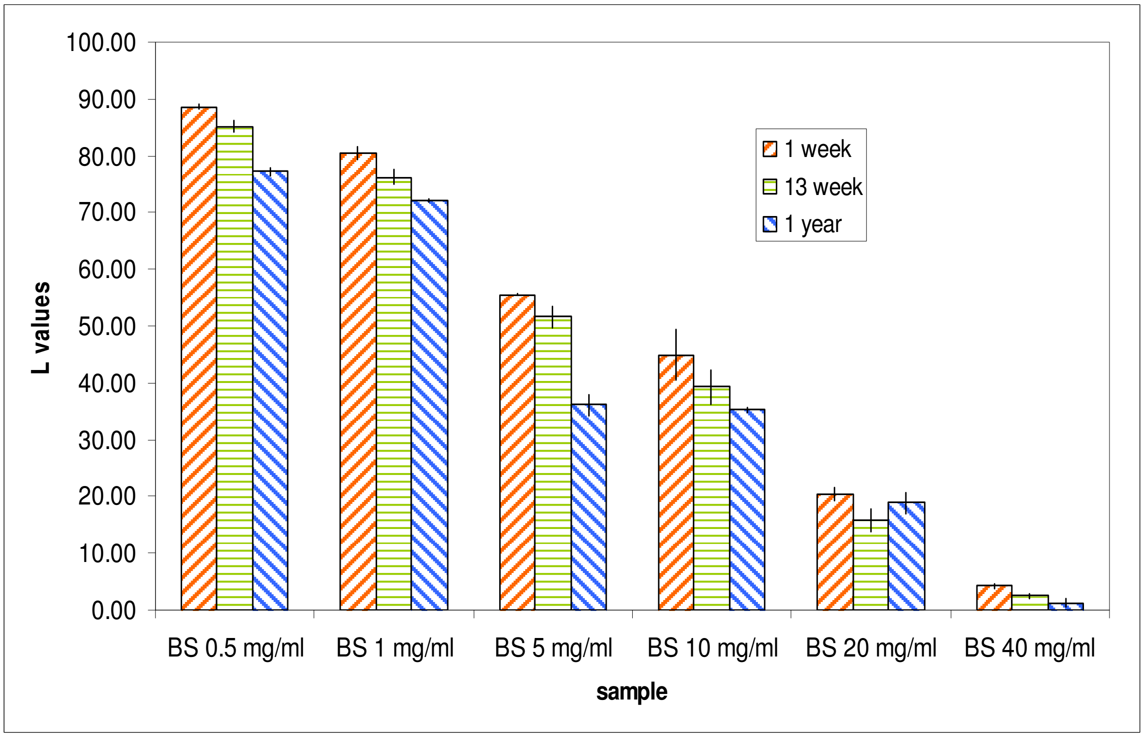

Fig. 81. Lightness values of Tx430 Black sorghum bran extracts (BS) at 1 and 13 weeks, and 1 year at different concentrations ($P< 0.05$).

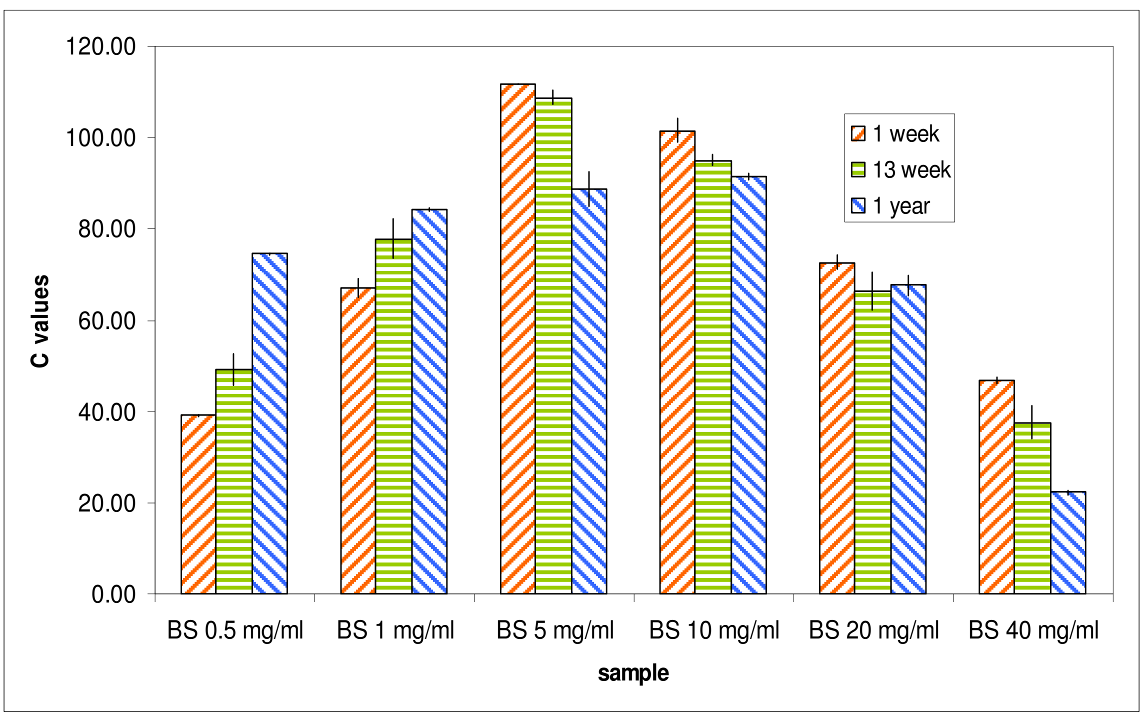

Fig. 82. Chroma values of Tx430 Black sorghum bran extracts (BS) at 1 and 13 weeks, and 1 year at different concentrations ($P< 0.05$).

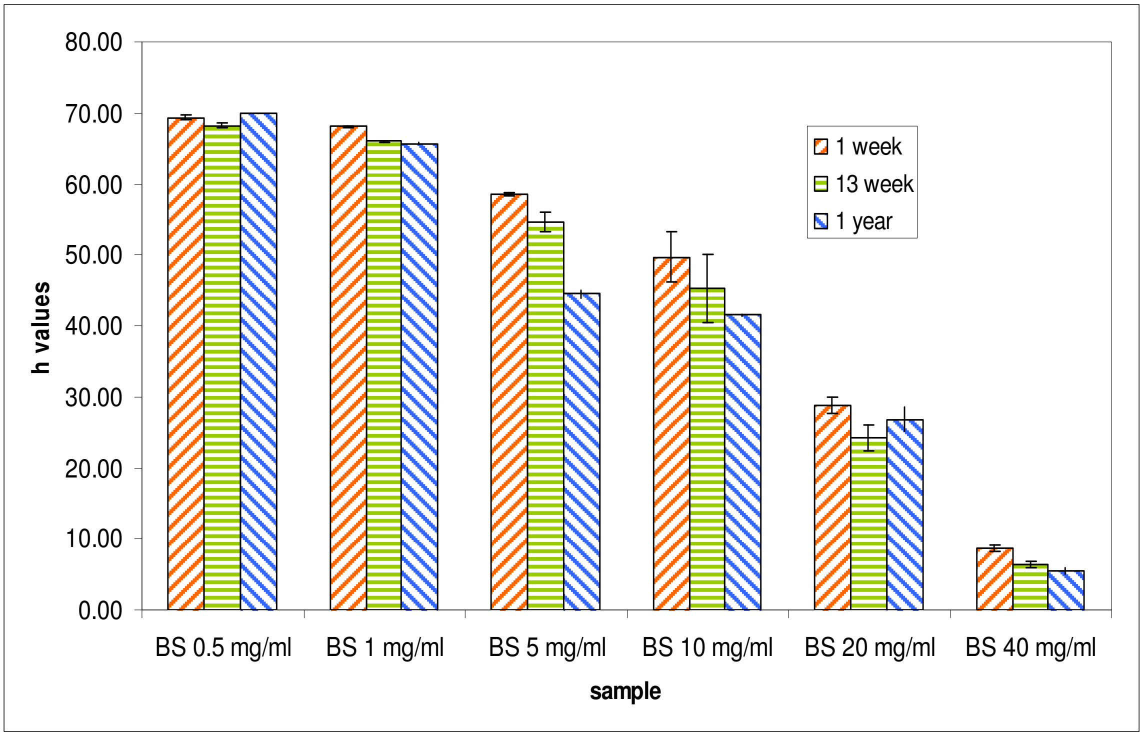

Fig. 83. Hue values of Tx430 Black sorghum bran extracts (BS) at 1 and 13 weeks, and 1 year at different concentrations (P< 0.05).

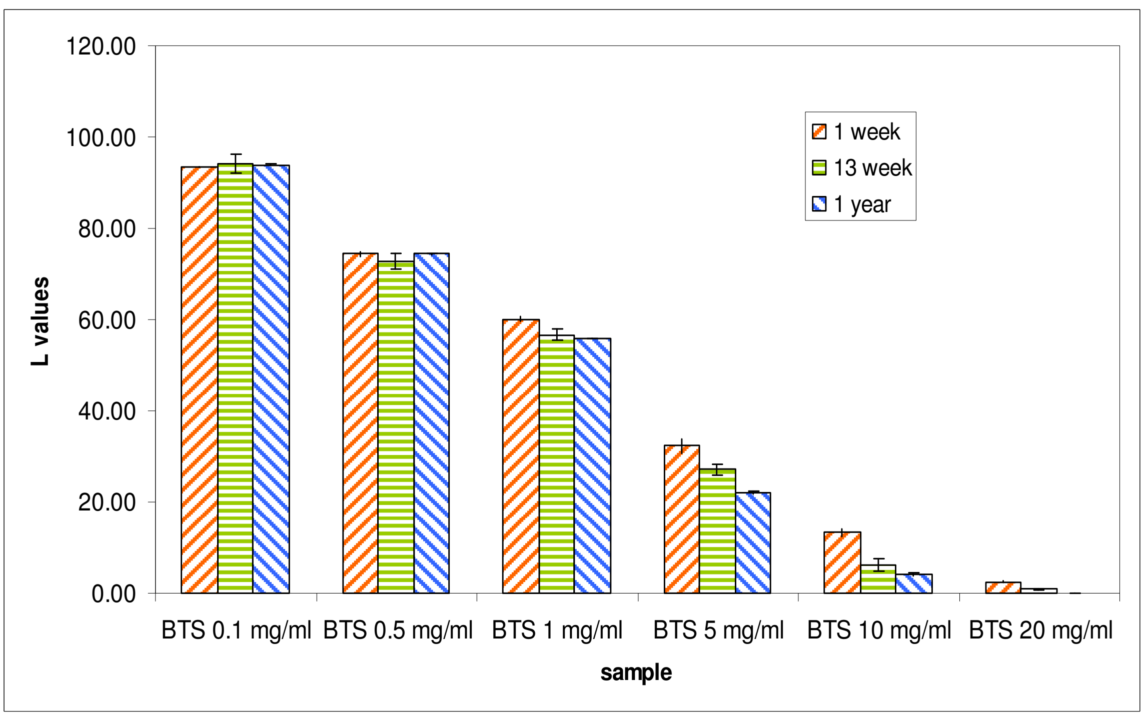

Fig. 84. Lightness values of Black PI Tall sorghum bran extracts (BTS) at 1 and 13 weeks, and 1 year at different concentrations (P< 0.05).

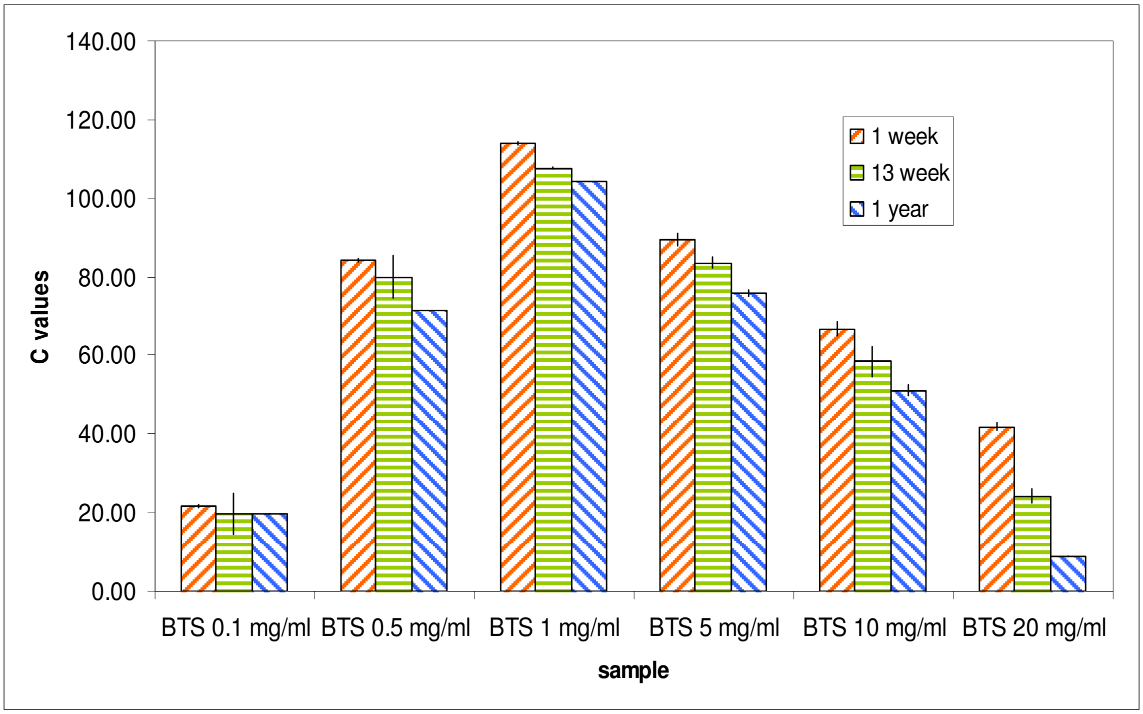

Fig. 85. Chroma values of Black PI Tall sorghum bran extracts (BTS) at 1 and 13 weeks, and 1 year at different concentrations ($P < 0.05$).

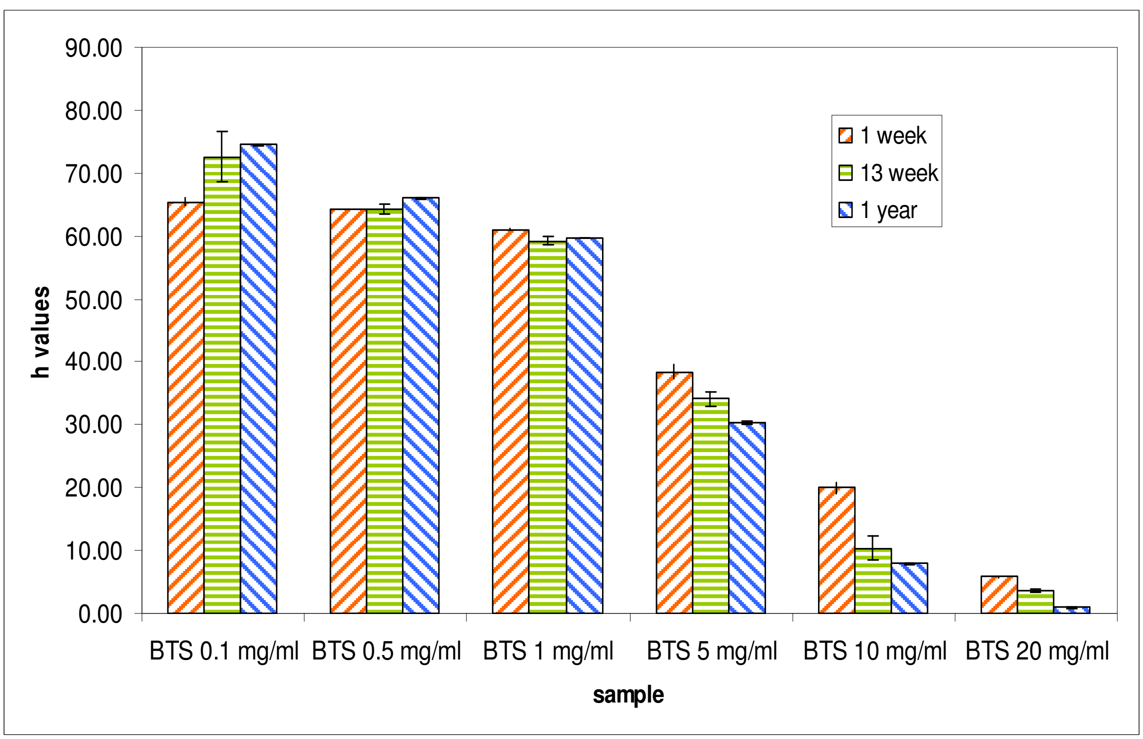

Fig. 86. Hue values of Black PI Tall sorghum bran extracts (BTS) at 1 and 13 weeks, and 1 year at different concentrations ($P < 0.05$).

APPENDIX E

STANDARD CURVES

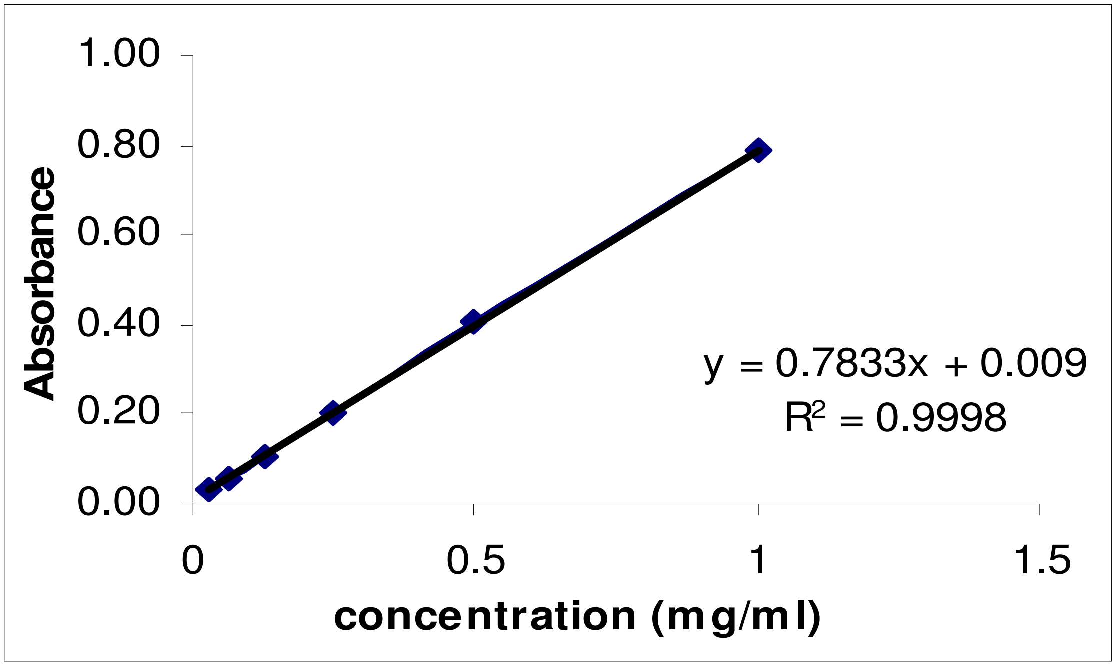

Fig. 87. Standard curve of Tx430 Black sorghum bran (ST) extracted with 0.5% citric acid in aqueous ethanol, measured at 485 nm.

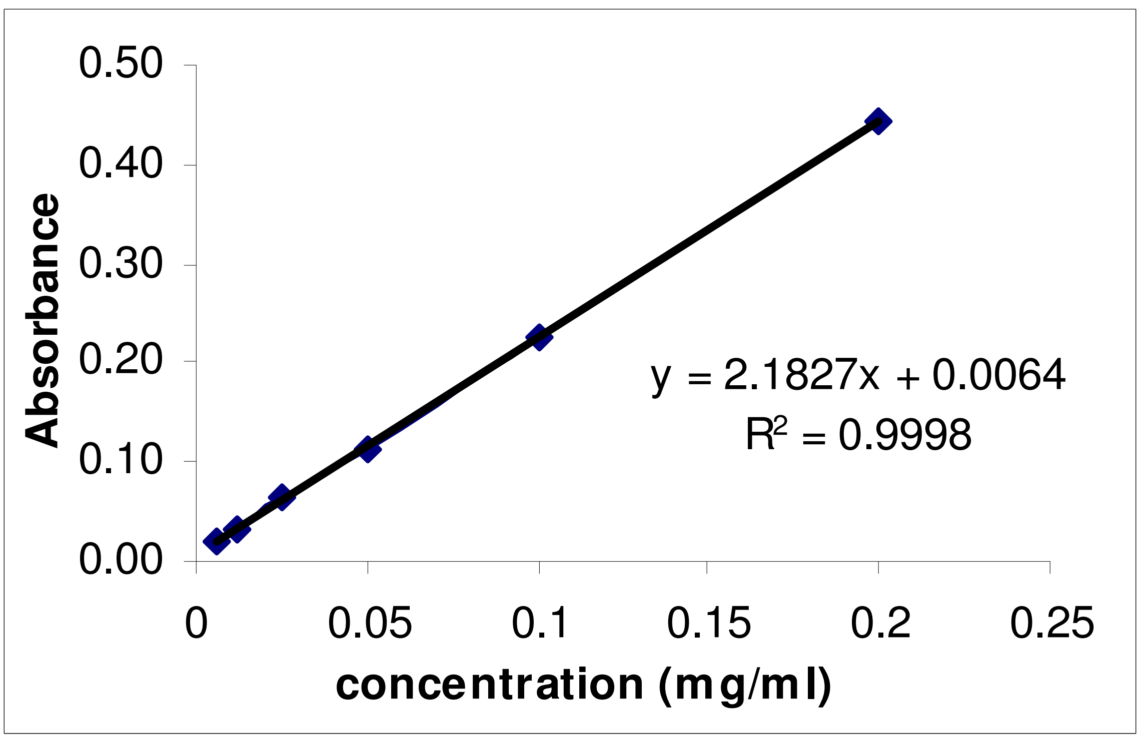

Fig. 88. Standard curve of Black PI Tall sorghum bran (BTS) extracted with 0.5% citric acid in aqueous ethanol, measured at 485 nm.

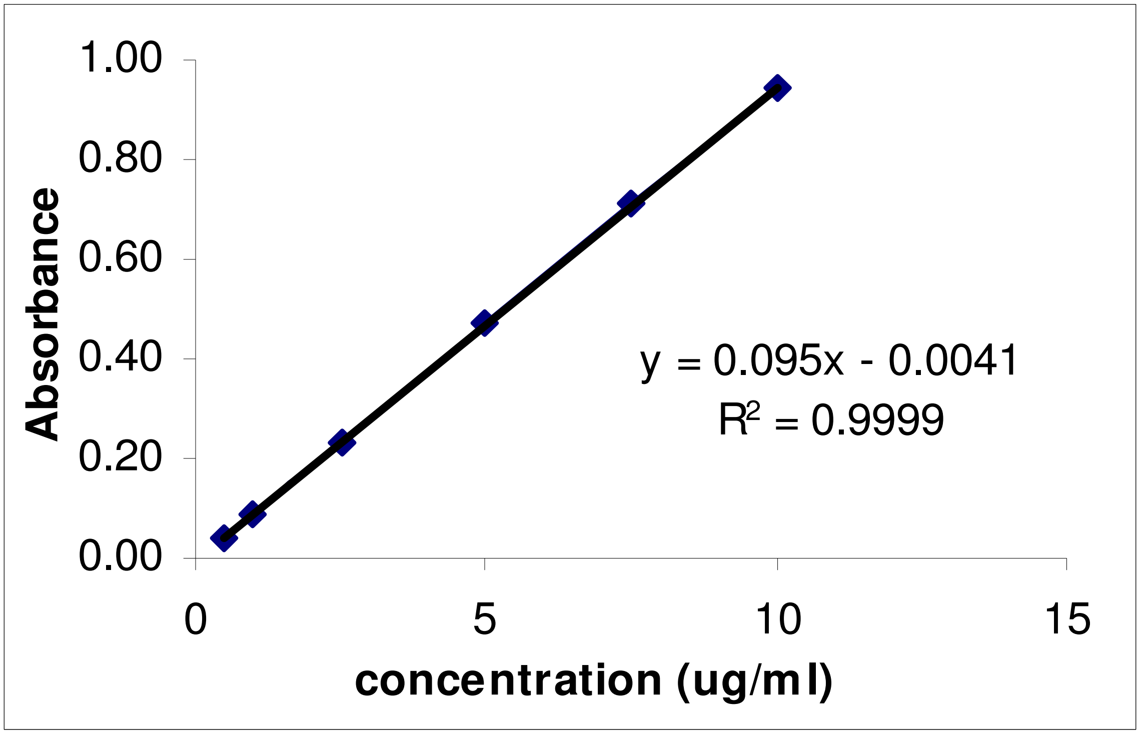

Fig. 89. Standard curve of purified luteolinidin extracted with 0.5% citric acid in aqueous ethanol, measured at 485 nm.

VITA

Ana Paola Cárdenas Hinojosa received her Bachelor of Science degree in food engineering from Instituto Tecnologico y de Estudios Superiores de Monterrey in 2004. She entered the Food Science program at Texas A&M University in August 2005 and received her Master of Science degree in December 2008. Her research interests include nixtamalization of corn, phytochemicals of sorghum and other cereal grains, extrusion of sorghum, inclusion of sorghum bran in bakery products.

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